The Pennsylvania State University

The Graduate School

Department of Bioengineering

ON-CHIP MICRODIALYSIS SYSTEM WITH FLOW-THROUGH GLUCOSE SENSING CAPABILITIES

A Thesis in

Bioengineering

by

Yi-Cheng Hsieh

© 2006 Yi-Cheng Hsieh

Submitted in Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy

December 2006
The thesis of Yi-Cheng Hsieh was reviewed and approved* by the following:

Jeffrey D. Zahn  
Assistant Professor  
Thesis Advisor  
Chair of Committee

Arnold A. Fontaine  
Senior Scientist

Nadine B. Smith  
Associate Professor

Michael V. Pishko  
Professor

Herbert H. Lipowsky  
Professor  
Head of the Department of Bioengineering

*Signatures are on file in the Graduate School
ABSTRACT

Microdialysis is a sampling technique based on controlling the mass transfer rate of different-sized molecules across a semipermeable membrane. Since the dialysis process does not change or affect the surrounding fluid, it is viewed as a tool for continuous monitoring of human metabolites for diabetes treatment. In diabetes treatment, microdialysis probes have been used as sampling systems coupled to a glucose biosensor but struggle to obtain high recoveries of analytes while the sampling housing, probes, and glucose sensors are fabricated as separate pieces and then assembled resulting in a large dead volume. An in-situ combination of a miniaturized microdialysis probe with an integrated glucose sensor allows both a high glucose recovery and frequent glucose sampling for continuous patient monitoring.

A microdialysis chip with flow-through electrochemical glucose sensing capabilities is presented here. The system was fabricated by bonding a 6 µm thick polycarbonate track-etch membrane with varying pore sizes onto microfluidic channels with the sensing electrodes patterned within the microchannels. A complete fabrication protocol for this device was developed with a non-leaking and reproducible bond between the microfluidic channels and the membrane. The integrated sampling and sensing components were both based on the same thin-film fabrication methods, which can reduce the dead volume and enhance microdialysis recovery with better time resolution.

As the first step toward the on-chip integration of a microdialysis system and a glucose sensor, impedance electrodes sputtered within the microchannels were used to characterize conductivity of a dialyzed phosphate buffered saline (PBS) solution, which is a
function of PBS concentration. The permeability of the membrane to the salt ions was obtained as $0.246 \, \mu m/s$ (15 nm diameter pores). Subsequently, experiments measuring PBS dialysis in the time-domain at 64.4% recovery were conducted.

Next glucose microdialysis with continuous sensing was demonstrated, the permeability of glucose to the polycarbonate membrane with 100 nm diameter pore size was obtained to be $5.44 \, \mu m/s$. Glucose recovery of 99% was observed using this microdialysis system at a perfusion flow rate of 0.5 $\mu l/min$. Experiments monitoring glucose concentration in the time-domain were also performed. The electrochemical sensing component was able to continuously track concentration changes in the reservoir. This system is expected to have the proper sensitivity to track physiologically relevant concentration changes of glucose (maximum change rate $\sim 4 \, mg/dl-min$ with periodicity of 1 hour or greater) with minimal lag time and amplitude reduction for continuous glucose monitoring for diabetes treatment.
# TABLE OF CONTENTS

LIST OF FIGURES ................................................................................................................... vii

LIST OF TABLES.................................................................................................................... x

ACKNOWLEDGEMENTS ....................................................................................................... xi

Chapter 1 Introduction ............................................................................................................. 1

Chapter 2 Background............................................................................................................. 4

2.1 Diabetes Mellitus ............................................................................................................. 4
   2.1.1 Insulin.................................................................................................................. 6
   2.1.2 Two Primary Types of Diabetes Mellitus ...................................................... 8
      2.1.2.1 Type 1 Diabetes Mellitus ...................................................................... 9
      2.1.2.2 Type 2 Diabetes Mellitus ...................................................................... 10
   2.1.3 Complications and Treatment ......................................................................... 11
   2.1.4 Glycemic Monitoring Devices on the Market............................................... 13
      2.1.4.1 Finger-Pricking Glucose Monitors...................................................... 13
      2.1.4.2 Continuous Glucose Monitoring Systems.......................................... 18
      GlucoWatch Biographer.................................................................................... 18
      Medtronic MiniMed CGMS Guardian ............................................................ 21
      GlucoDay Microdialysis System ....................................................................... 22
   2.2 Microdialysis .............................................................................................................. 25
      2.2.1 Development of Microdialysis......................................................................... 25
      2.2.2 Basic Principles .................................................................................................. 27
      2.2.3 Quantitative Microdialysis Models.................................................................. 30
      2.2.4 Factors Affecting Recovery ............................................................................. 33
         2.2.4.1 Perfusate Flow Rate............................................................................... 33
         2.2.4.2 Dialysis Membrane Area and Membrane Material............................ 34
         2.2.4.3 Diffusion Coefficient............................................................................. 34
      2.2.5 Calibration Techniques..................................................................................... 35
         2.2.5.1 Perfusate Flow Rate Variation ............................................................. 36
         2.2.5.2 No-net Flux Method.............................................................................. 36
         2.2.5.3 Retrodialysis ............................................................................................ 37
      2.2.6 Microdialysis for the Management of Diabetes Mellitus............................. 38
      2.2.7 New Frontier – Microdialysis Chips............................................................... 39

Chapter 3 On-chip Microdialysis System with Impedance Sensing Capability......... 43

3.1 Theory ............................................................................................................................ 43
   3.1.1 Thin Film Model................................................................................................ 44
   3.1.2 Mass Transfer Prediction ................................................................................. 46
   3.1.3 The Concept of Electrolytic Conductivity..................................................... 49
LIST OF FIGURES

Figure 2-1: Error Grid Analysis. (From: D. A. Gough and E. L. Botvinick, 1997) ........ 16
Figure 2-2: Bland-Altman Analysis (From: A. Kerssen et al. 2005) ................. 17
Figure 2-3: The GlucoWatch G2 Biographer ..................................................... 19
Figure 2-4: Representative GlucoWatch® biographer results compared to blood glucose measurements for the clinical setting trial ......................... 20
Figure 2-5: The Medtronic MiniMed CGMS Guardian ....................................... 21
Figure 2-6: In vivo glucose sensing in a type 1 diabetic patient using the Medtronic-MiniMed Continuous Glucose Monitoring System (solid line), compared to intermittent blood glucose self-testing with capillary blood samples (squares) .... 22
Figure 2-7: The GlucoDay Microdialysis System ............................................... 23
Figure 2-8: In vivo glucose sensing using the GlucoDay in a type 1 diabetic for 24 hours, compared to intermittent blood glucose self-testing with capillary blood samples ................................................................. 24
Figure 2-9: Concentric Microdialysis Probe ...................................................... 28
Figure 2-10: Different probe designs of commercially available microdialysis probes .... 28
Figure 2-11: CMA Microdialysis Probes ......................................................... 29
Figure 2-12: A schematic of the cross-section of a microdialysis probe .......... 32
Figure 2-13: Regression Analysis for the No-net Flux Method ......................... 37
Figure 2-14: Microdialysis Chips ..................................................................... 40
Figure 3-1: Thin-film model ............................................................................. 44
Figure 3-2: Schematic of the Mass Transfer of the On-chip Microdialysis System .... 46
Figure 3-3: Concept of mixing ....................................................................... 52
Figure 3-4: Schematic of the stacked microdialysis system with impedance sensing electrodes ................................................................. 53
Figure 3-5: Mask for impedance sensing electrodes ........................................ 54
Figure 3-6: Mask for SU-8 microfluidic channel with impedance sensing electrodes ..... 54

Figure 3-7: SEM pictures showing the bonding between the SU-8 layer and the polycarbonate membrane. (Top) a patterned area showing the channel features and bonded membrane. (Bottom) a cleaved slide showing a continuous bond along the length of the field SU-8................................................................. 58

Figure 3-8: Complete microdialysis chip fabrication procedures............................... 59

Figure 3-9: Mask for the PDMS reservoir channel of impedance sensing .................. 61

Figure 3-10: Alignment of impedance electrodes, SU-8 channel and PDMS reservoir channel under microscope................................................................. 62

Figure 3-11: Photo of a complete microdialysis system with impedance sensing capabilities................................................................................................................. 63

Figure 3-12: The geometry of the mixer .................................................................. 63

Figure 3-13: Experimental setup for achieving time varying reservoir concentrations..... 65

Figure 3-14: The LabView program to control flow rates of two syringe pumps and to collect impedance data. Notice that the two syringe pump flow rates are 180° out of phase to produce a sinusoidally changing reservoir concentration.................. 66

Figure 3-15: Calibration of PBS conductance as a function of PBS concentration ........ 68

Figure 3-16: PBS microdialysis recovery.................................................................... 69

Figure 3-17: Time domain of step PBS dialysis at a perfusion flow rate of 0.1 µL/min ......................................................................................................................... 71

Figure 3-18: The response of sinusoidal input demonstrated with the impedance microdialysis system......................................................................................... 72

Figure 3-19: Computational simulation using Comsol .............................................. 73

Figure 4-1: The three-electrode voltammetric configuration...................................... 81

Figure 4-2: Schematic of the stacked microdialysis system with glucose sensing electrodes.............................................................................................................. 82

Figure 4-3: Mask for glucose sensing electrodes....................................................... 83

Figure 4-4: Mask for SU-8 microfluidic channel of glucose sensing.......................... 84

Figure 4-5: Designs for PDMS reservoir/mixer channel layer..................................... 85
Figure 4-6: Pictures of an actual glucose microdialysis chip................................. 86

Figure 4-7: Experimental setup for glucose sensing........................................ 88

Figure 4-8: A representative graph for a glucose concentration of 265 mg/dL recorded from the CH Instruments packaged software............................. 89

Figure 4-9: Glucose concentration v.s. Oxidation current.................................. 91

Figure 4-10: Glucose microdialysis recovery.................................................... 94

Figure 4-11: Flow rate control for the step response........................................ 95

Figure 4-12: Time domain of step glucose dialysis at a perfusion flow rate of 0.5 μL/min.................................................................................................................. 96

Figure 4-13: Flow rate control for the sinusoidal input..................................... 97

Figure 4-14: The response of sinusoidal input demonstrated with the glucose microdialysis system................................................................. 98
LIST OF TABLES

Table 2-1: Comparisons of Type 1 and Type 2 Diabetes Mellitus ........................................ 11

Table 2-2: Comparison of Glucose Monitors on the Market .................................................. 15

Table 3-1: Photolithography procedures for Shipley 1818 ..................................................... 55

Table 3-2: Photolithography procedures for SU-8 2010 of 15 µm depth ............................. 57

Table 3-3: Photolithography procedures for SU-8 2010 of 20 µm depth .............................. 60

Table 3-4: PBS microdialysis results ...................................................................................... 70

Table 4-1: Glucose microdialysis results .............................................................................. 93
ACKNOWLEDGEMENTS

I would like to thank my advisor, Dr. Jeffrey D. Zahn, for his help and instruction, my family and friends, for their support and encouragements.

This work is funded by American Diabetes Association.
Chapter 1

Introduction

Diabetes mellitus is a clinically heterogeneous group of disorders characterized by elevated blood glucose levels resulting from deficiency in insulin secretion or insulin resistance, or a combination of both. The blood glucose concentration is regulated within a narrow range, 3.5 to 5 mM, in normal healthy people, while the level in diabetic patients varies considerably, between 1 to 30 mM. In diabetics, the consistently high glucose levels results in long-term complications including retinopathy, nephropathy and neuropathy which often leads to amputation of extremities. Studies have shown that intensive glycemic control can reduce the long-term complication dramatically.

However, one of the disadvantages of the glycemic control was a tripling of the risk of hypoglycemic incidences highlighting the need for a feedback controlled insulin infusion system or ‘artificial pancreas’. One of the critical components of such a system is a continuous microdialysis based glucose sensing system.

Commercially available microdialysis probes have been used for diabetes treatment as a continuous monitoring system coupled to a glucose sensor but struggle to obtain high recoveries of analytes while the sampling probes and glucose sensors are fabricated as separate pieces and then assembled resulting in a large dead volume.

In this work, a novel on-chip microdialysis system with glucose sensing capabilities is presented, which solves the problems mentioned above. The device is based on thin-film fabrication, which is easily integrated with in-situ biosensors, and direct polymer bonding on
microfluidic channels designed for direct contact between the dialysis membrane and tissue of interest.

In Chapter 2, some background information about diabetes mellitus is discussed, such as insulin, classification, complications and treatment followed by the introduction of microdialysis, which has been used as a glucose continuous monitoring tool in diabetics. Commercially available microdialysis probes, the concept of this technique and our vision to make it on-chip are also introduced here.

In Chapter 3, as a first step towards the integration of a microdialysis system and a biosensor, continuous sensing was demonstrated by measuring changes in solution conductivity in a dialyzed solution. An on-chip microdialysis system with gold sensing electrodes was developed and used to determine fluid electrical resistance from a dialyzed phosphate buffered saline (PBS) solution. The electrodes characterized solution conductivity as a function of PBS concentration. The permeability of the polycarbonate membrane (15 nm diameter pore size) to the salt ions was obtained as 0.246 µm/s. Subsequently, experiments measuring PBS dialysis at 64.4% recovery in the time-domain were conducted. The PBS concentration of the reservoir was changed in both a step response and sinusoidally with an 800 second period. The subsequently measured impedance indicates that the system is able to continuously track concentration changes.

In Chapter 4, an electrochemical glucose sensor was integrated with the microdialysis system. Instead of gold (Au) impedance electrodes, platinum (Pt) and silver/silver chloride (Ag/AgCl) electrodes were used to determine the glucose concentration of the dialysate after passing through the microdialysis area. The permeability was obtained as 5.44 µm/s for a polycarbonate membrane with 100 nm diameter pore size and was in good agreement with
the theory. Glucose dialysis experiments were conducted in the time domain at recovery of 99%. The glucose sensing component was successfully integrated with the microdialysis chip and was able to continuously track glucose concentration change in vitro. This system is expected to have the proper sensitivity to track physiologically relevant concentration changes of glucose with minimal lag time and amplitude reduction for continuous glucose monitoring for diabetes treatment.

Finally, conclusions and future work are discussed in Chapter 5. The complete fabrication protocol for the on-chip microdialysis system was developed. In order to create a reservoir environment with the concentration continuously being changed for testing the time resolution of the device, an experimental set-up was constructed to test the microdialysis system sufficiently. In the future, different types of semipermeable membrane can be integrated with the microdialysis system. Some adjustments for the glucose sensor are considered, such as enzyme immobilization and built-in reference electrode. The system should be used for biological studies in vivo such as implanting the probe in a diabetic animal model. Additional integrations with micropumps, microvalves and other components are possible as well, leading to more self-contained µTAS systems.
Chapter 2

Background

In this chapter, diabetes mellitus, its complications and treatments are introduced. Studies have shown that the long term complications of the disease can be reduced dramatically by tight glycemic control, which can be accomplished by insulin injection and continuous glucose monitoring. One method to achieve continuous glucose monitoring is microdialysis. Microdialysis is a sampling technique first used in neuroscience to monitor neurotransmitters in the brain. It has been found useful for glucose monitoring in diabetics as well. The details about the microdialysis technique are presented in the second part of this chapter.

2.1 Diabetes Mellitus

Diabetes mellitus is a clinically heterogeneous group of disorders characterized by elevated blood glucose levels resulting from deficiency in insulin secretion or insulin resistance, or a combination of both. The term, “diabetes”, comes from the Greek term meaning flow of fluid through a siphon because the disease involves discharge of excessive urine and “mellitus” means sweet or honey in Latin.

In normal healthy people the blood glucose concentration is regulated within a narrow range, 3.5 to 5 mM (63 to 90 mg/dL), while the level in diabetic patients varies considerably, between 1 to 30 mM (18 to 540 mg/dL). The hormone, insulin, produced in the β cells within the islets of Langerhans of the pancreas regulates this metabolism. In the
absence of insulin or without the correct response to insulin, fats undergo beta-oxidation, amino acids go through gluconeogenesis to become glucose, and glycogen stores are also turned back into glucose. As a result, people with diabetes usually have an elevated blood glucose concentration.

The current methods for diagnosing diabetes mellitus are an oral glucose tolerance test and fasting hyperglycemia. The diagnostic criteria are that the fasting plasma glucose level is higher than 126 mg/dL or the plasma glucose level is higher than 200 mg/dL at two hours after a 75 g oral glucose challenge[1]. Both methods need to be confirmed by repeat testing on a different day. Nowadays, the oral glucose tolerance test, however, is not recommended in clinical practice because of the difficulties inherent in performing the test. For people already with symptoms of diabetes, such as polyuria, polydipsia and polyphagia, the disease can be diagnosed by a casual plasma glucose level higher than 200 mg/dL.

Another way to measure blood glucose level for diabetics is called hemoglobin A1C test or HbA1C test. The test reveals the average blood glucose level of the last three months. Glycosylation is used to describe the process in which glucose in the blood stream attaches to the hemoglobin within red blood cells. The higher blood glucose, the more glucose which attaches to hemoglobin. Once the hemoglobin is glycosylated, the glucose stays attached for the lifetime of the red blood cells, which is about 120 days. This test takes a small sample of blood and measures the amount of attached glucose to the hemoglobin. Results are given in percentages. The goal for people with diabetes is less than 7 percent meaning the blood glucose levels rarely exceed 150 mg/dL during the previous three months.

According to the American Diabetes Association, there are 18.2 million people in the United States, or 6.3% of the population, who have diabetes. Four out of ten people with
diabetes develop complications, including: blindness, kidney disease, nerve damage which often leads to amputation of extremities, heart attack and stroke. The total estimated cost of diabetes to the USA in 2002 was $132 billion and the number of diabetics is expected to rise 44% in the next twenty years[2].

2.1.1 Insulin

Insulin is a very large polypeptide with a molecular weight of 5.8 kDa. It consists of two straight peptide chains which are linked together by disulfide bonds. Insulin is synthesized and secreted by the beta cells of the islets of Langerhans in the pancreas. A specific transmembrane protein, Glut-2, concentrated in the microvilli of the canaliculi between beta cells is the transporter to facilitate diffusion of glucose into the beta cells, which maintain the glucose concentration in the beta cells equal to that in the interstitial fluid. Therefore, insulin secretion can be triggered within a very short time when the plasma glucose level is above normal. Individual beta cells have different sensitivities to glucose, and not all of them respond at one time. Dispersed or gap junction blocked beta cells secrete much less insulin, which demonstrates the importance of cell-to-cell signaling in insulin secretion.

Once insulin arrives at the target cells (mainly muscle and adipose cells), it binds with and activates a membrane receptor. The receptor is a combination of four separate subunits joined by disulfide linkages, two completely extracellular alpha subunits and two transmembrane beta subunits with two ends protruding into the cytoplasm. As soon as insulin binds with the alpha subunits, the protruding portions of beta subunits become
autophosphorylated and activate the intercellular signal transduction. Subsequently, the activated receptor causes the membrane to become highly permeable to glucose by opening the glucose transporter protein, Glut-4, which is specifically expressed in muscle and adipose tissue, from a cytoplasm pool of vesicles to the plasma membrane.

One of the most important actions of insulin is to put glucose absorbed after meals into storage in the liver in the form of glycogen. Glycogen can subsequently be broken down back to glucose and released into the blood to maintain the blood glucose level when needed. Insulin enhances the uptake of glucose from the blood into the liver cells by inducing glucokinase, an enzyme, which catalyzes the phosphorylation of the incoming glucose. Once phosphorylated, the glucose is temporarily trapped inside the liver cells and cannot diffuse back through the membrane. Insulin promotes the storage of glycogen by increasing the activity of the glycogen synthesis enzymes. In addition, it inhibits gluconeogenesis by decreasing the quantities and activities of the enzymes required. Insulin facilitates glucose transport into muscle cells and adipose tissue. When excess glucose is transported into the muscle cells, most of the glucose is converted to glycogen and stored in the cells for later energy use. Within adipose tissue, the transport of glucose is essential for providing the glycerol portion of the fat molecule for deposition of fat in these cells.

Insulin increases the utilization of glucose and decreases the utilization of fat. Even though the effects of insulin on fat metabolism are not as visible and acute as that on carbohydrate metabolism, it is very important. Over the long term a lack of insulin causes atherosclerosis of blood vessels. Insulin helps store fat in adipose cells by inhibiting the action of hormone-sensitive lipase, an enzyme which causes the hydrolysis of triglycerides into fatty acids, and by promoting the deposition of circulating fatty acids into adipose tissue.
by activating the necessary enzymes. A major consequence is a reduced concentration of free fatty acid and ketoacid in the blood plasma, which has made insulin to be considered the major and perhaps the only antiketogenic hormone. Within muscle cells, the overall effects of insulin reinforce the principle that insulin promotes the utilization of glucose and the storage of fatty acids. The same principle also applies on the liver, since insulin is antiketogenic and lipogenic. The net overall effect of insulin is to enhance storage and to block mobilization and oxidation of fatty acids.

Even though the mechanism is not well understood, insulin also promotes protein formation and prevents the catabolism of proteins, thus it decreases the release rate of amino acids from cells. In the absence of insulin, virtually all protein storage comes to a complete halt.

2.1.2 Two Primary Types of Diabetes Mellitus

In the mid-1930’s, Himworth proposed that there were at least two types of diabetes mellitus[3]. His observations were confirmed by Bornstein and Lawrence in the 1950’s[4]. The disease is therefore divided into two distinct types: type 1 and type 2 diabetes. These two types of diabetes have very different etiologic factors and clinical presentations. The classification system also includes two other categories termed “gestational diabetes mellitus” and “other types of diabetes mellitus”. The former occurs in about 2%-5% of all pregnancies[1] and is usually temporary. However, women with GDM have higher risk for developing type 2 diabetes after pregnancy later in their lives. The forth type of diabetes comprises other rare causes of the disease that do not fit into the classical definition type 1,
type 2 or gestational diabetes. Table 2-1 summarizes many of the differences between type 1 and type 2 diabetes.

2.1.2.1 Type 1 Diabetes Mellitus

Type 1 diabetes has a complete lack of insulin, which can be life-threatening. Type 1 diabetics comprise about 5%~10% of cases in the diabetes mellitus syndrome. The onset of severe symptoms is usually abrupt and occurs at a young age, so that it used to be called “juvenile-onset diabetes”. The disease results from autoimmune destruction of pancreatic beta cells. For people with type 1 diabetes, their immune system fails to recognize the beta cells as “self” and attacks them with antibodies and white blood cells resulting in beta-cell destruction, which leads to loss of insulin secretion and absolute insulin deficiency (insulinopenia). Exogenous insulin is the only treatment for type 1 diabetics to sustain their life[1, 5].

Insulin deficiency reduces the utilization of peripheral glucose, while hepatic glucose production, proteolysis and lipolysis are uninhibited in the absence of insulin. The uninhibited hepatic glucose production from glycogenolysis and gluconeogenesis worsen the hyperglycemia. If the hyperglycemia exceeds one’s renal threshold for glucose, the reabsorption becomes saturated and some glucose is excreted in the urine (glucosuria), which subsequently leads to reduced water reabsorption and large volumes of urine (polyuria). Lipolysis produces free fatty acids for ketone body production. As a result, people with type 1 diabetes are prone to ketosis. Because many ketones are strong acids,
metabolic acidosis is induced. If the diabetic ketoacidosis remains untreated, it can cause coma or even death.

2.1.2.2 Type 2 Diabetes Mellitus

Type 2 diabetes comprises about 90%~95% of cases in the diabetes mellitus syndrome. It results from insulin resistance in the muscle, liver and adipose tissue. The symptoms are not as severe as type 1 because insulin is still present, and in some cases the insulin level may be even higher than normal. Type 2 diabetes is often unrecognized and untreated in the early stage because of many patients being asymptomatic. The etiology of type 2 diabetes is still unclear. It is strongly suggested to have a genetic association because the familial pattern of occurrence is often observed. Other factors including obesity, increasing age, high caloric intake, sedentary lifestyle and low birth weight have also been identified.

A certain amount of glucose metabolism is still performed since insulin is not completely deficient. Nevertheless, the overall metabolism is not normal and people with type 2 diabetes develop an assortment of diabetes-related problems. Type 2 diabetes is a chronic and progressive disease. Patients are often treated by adjustment in diet, exercise and by weight loss. Close glycemic control is important to prevent the disease from progressing and to reduce mortality and morbidity. Patients may require insulin for correction of fasting hyperglycemia but are usually not prone to ketosis. As type 2 diabetes progresses, a patient can become absolute insulin deficient and often needs exogenous insulin injections to maintain blood glucose levels[1, 6].
2.1.3 Complications and Treatment

In diabetics, the consistently high glucose levels results in long-term complications including retinopathy, nephropathy and neuropathy which often leads to amputation of extremities. Retinopathy includes various disorders of the retina that affects vision, which is usually due to small vessel damage next to the retina caused by diabetes. If left untreated, it may result in blindness. Diabetic nephropathy is the leading cause of end stage renal disease and the prime cause for dialysis in the developed countries. When the kidney fails completely, dialysis is absolutely necessary and kidney transplantations must be considered. Peripheral neuropathy is the most common type of diabetic neuropathy which damages the nerves of the limbs, especially the feet. The symptoms of peripheral neuropathy comprise

Table 2-1: Comparisons of Type 1 and Type 2 Diabetes Mellitus

<table>
<thead>
<tr>
<th></th>
<th>Type 1 Diabetes Mellitus</th>
<th>Type 2 Diabetes Mellitus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Other Names</td>
<td>Juvenile-Onset Diabetes, Insulin-Dependent Diabetes (IDDM)</td>
<td>Adult-Onset Diabetes, Non-Insulin-Dependent Diabetes (NIDDM)</td>
</tr>
<tr>
<td>Pathogenesis</td>
<td>Autoimmune destruction of beta cells leads to severe insulin deficiency</td>
<td>Insulin resistance of muscles, liver and adipose tissue. The real cause remains unclear</td>
</tr>
<tr>
<td>Percentage</td>
<td>5-10 %</td>
<td>90-95 %</td>
</tr>
<tr>
<td>Age of Onset</td>
<td>usually &lt; 20 years old</td>
<td>usually &gt; 40 years old</td>
</tr>
<tr>
<td>Body Fat</td>
<td>Normal</td>
<td>usually obese</td>
</tr>
<tr>
<td>Ketosis</td>
<td>Common</td>
<td>Not prone to</td>
</tr>
<tr>
<td>Familiar History</td>
<td>No</td>
<td>Strong association</td>
</tr>
<tr>
<td>Exogenous Insulin</td>
<td>Required</td>
<td>Not in the beginning. But as the diseases progresses, insulin is frequently needed for correction of blood glucose levels</td>
</tr>
</tbody>
</table>
numbness, slow reflex and weak muscles. Because of the loss of sensation, injuries or diabetic ulcers may be unnoticed and often become infected. If the injuries are not treated in time, the infection can spread and the foot or lower leg may require amputation.

Studies have shown that the inability to secret epinephrine and glucagons in some type 1 diabetics patients can place them at risk for developing clinical hypoglycemia because the body fails to have hypoglycemia-related autonomic warning symptoms[7, 8]. For type 1 diabetics, nocturnal hypoglycemia is common and usually asymptomatic. Almost 50% of all episodes of severe hypoglycemia occur at night during sleep[9]. It can cause convulsions, coma and have been implicated as a precipitating factor in cardiac arrhythmias resulting in sudden death. The mood and well-being may be affected on the day after an episode of nocturnal hypoglycemia. Recurrent episodes may impair cognitive function, and perhaps lead to the development of "hypoglycemia unawareness", which means difficulty recognizing early warning symptoms of hypoglycemia.

The 10 year Diabetes Control and Complications Trial (DCCT)[10] was designed to determine whether intensive glycemic control affects the complications in type 1 diabetes mellitus. More than 1400 patients with type 1 diabetes participated in this project. These patients were requested to have three or more insulin injections per day or insulin pump therapy guided by frequent self blood glucose monitoring and their hemoglobin A1c levels were taken monthly in order to keep the blood glucose levels as close as possible to the nondiabetic range. The therapy was also adjusted frequently in response to every patient’s current situation. The results showed that intensive therapy reduced the development of diabetic long-term complications. Retinopathy was reduced by 76%. Nephropathy was reduced by 69%. Neuropathy was reduced by 60%. This study reinforced the role of
intensive insulin therapy for glycemic control. Results from the United Kingdom Prospective Diabetes (UKPDS) have also demonstrated the benefits of intensive insulin therapy for type 2 diabetics[6, 11].

However, one of the disadvantages of the intensive glycemic control was a tripling of the risk of hypoglycemic incidences highlighting the need for a feedback controlled insulin infusion system or ‘artificial pancreas’. One of the critical components of such a system is a continuous microdialysis based glucose sensing system.

2.1.4 Glycemic Monitoring Devices on the Market

It has been well established that tight glycemic control can dramatically reduce the long term complications in diabetes. In order to keep blood glucose levels as close to normal as possible, self-monitoring of blood glucose levels is highly recommended for diabetes care. This has led to a variety of glucose monitoring devices.

2.1.4.1 Finger-Pricking Glucose Monitors

Self-blood glucose monitoring (SBGM) can be achieved using a portable glucose monitor available in most pharmacies. The kit contains a glucose meter and its own reagent strips. Usually a drop of blood obtained through finger prick is placed on the reagent strip. A finger prick can be done with a small lancet fixed in a spring-loaded device that punctures the fingertip quickly. The strip is then placed in the blood glucose monitor, which reads the blood glucose level usually through enzyme linked electrochemistry.
There are many types of monitors on the market today, ranging in price, ease of use, sample size, portability, and length of testing time. Each monitor requires its own test strips and usually provides results within a couple of seconds. Table 2-2 provides basic information for six different kinds of glucose monitor available on the market. The smallest blood volume needed is 0.3 µL and it can give readings in 5 seconds. The reading range is between 20-500mg/dL (1.1mM-27.8mM) and up to 600mg/dL (33.3mM) for some of them. These monitors are usually evaluated by error grid analysis and/or Bland-Altman to determine their accuracy[12].
<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Lifescan</th>
<th>Lifescan</th>
<th>BD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meter</td>
<td>One Touch Ultra2</td>
<td>One Touch Ultrasmart</td>
<td>BD logic</td>
</tr>
<tr>
<td>Technology</td>
<td>Electrochemical</td>
<td>Electrochemical</td>
<td>Electrochemical</td>
</tr>
<tr>
<td>Overall Size</td>
<td>7.9x5.7x2.3 cm 3.12x2.25x0.9 inch</td>
<td>9.6x5.8x2.3 cm 3.8x2.3x0.9 inch</td>
<td>9.1x5.8x2.3 cm 3.6x2.3x0.9 inch</td>
</tr>
<tr>
<td>Weight</td>
<td>42.5 gram 1.5 ounces</td>
<td>80 gram 2.8 ounces</td>
<td>75 gram 2.65 ounces</td>
</tr>
<tr>
<td>Testing Time</td>
<td>5 seconds</td>
<td>5 seconds</td>
<td>5 seconds</td>
</tr>
<tr>
<td>Sample Size</td>
<td>1 µL</td>
<td>1 µL</td>
<td>0.3 µL</td>
</tr>
<tr>
<td>Download Capability</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Reported Result Range</td>
<td>20 - 600 mg/dL</td>
<td>20 - 600 mg/dL</td>
<td>20 - 600 mg/dL</td>
</tr>
<tr>
<td>Memory</td>
<td>500 test results</td>
<td>&gt;3000 test results</td>
<td>250 test results</td>
</tr>
<tr>
<td>Cost of Meter</td>
<td>$69.99/100 strips</td>
<td>$84.39/100 strips</td>
<td>$34.99</td>
</tr>
<tr>
<td>Cost of Strips</td>
<td>$94.99/100 strips</td>
<td>$94.99/100 strips</td>
<td>$89.99/100 strips</td>
</tr>
<tr>
<td>Features</td>
<td>Fast reading time. Easy-to-read screen. After or before meal flag feature available</td>
<td>Fast reading time. Input daily activities. Chart, graph and track trends. After or before meal flag feature available</td>
<td>Thinnest lancets. Small sample needed. Fast reading time. 7 or 14 days average.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>TheraSense</th>
<th>TheraSense</th>
<th>Roche</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meter</td>
<td>Freestyle Freedom</td>
<td>Freestyle Flash</td>
<td>Accu-Check Aviva</td>
</tr>
<tr>
<td>Technology</td>
<td>Electrochemical</td>
<td>Electrochemical</td>
<td>Electrochemical</td>
</tr>
<tr>
<td>Overall Size</td>
<td>8.4x5.1x1.6 cm 3.3x2x0.63 inch</td>
<td>7.6x4.1x2 cm 3.0x1.6x0.8 inch</td>
<td>9.4x5.3x2.2 cm 3.7x2.1x0.9 inch</td>
</tr>
<tr>
<td>Weight</td>
<td>40 gram 1.43 ounces</td>
<td>40 gram 1.4 ounces</td>
<td>60 gram 2.1 ounces</td>
</tr>
<tr>
<td>Testing Time</td>
<td>5 seconds</td>
<td>7 seconds</td>
<td>5 seconds</td>
</tr>
<tr>
<td>Sample Size</td>
<td>0.3 µL</td>
<td>0.3 µL</td>
<td>0.6 µL</td>
</tr>
<tr>
<td>Download Capability</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Reported Result Range</td>
<td>20 - 500 mg/dL</td>
<td>20 - 500 mg/dL</td>
<td>20 - 500 mg/dL</td>
</tr>
<tr>
<td>Memory</td>
<td>250 test results</td>
<td>250 test results</td>
<td>500 test results</td>
</tr>
<tr>
<td>Cost of Meter</td>
<td>$74.99</td>
<td>$74.99</td>
<td>$74.99</td>
</tr>
<tr>
<td>Cost of Strips</td>
<td>$94.99/100 strips</td>
<td>$94.99/100 strips</td>
<td>$97.99/100 strips</td>
</tr>
<tr>
<td>Features</td>
<td>Fast reading time. Ability to fill strip from both sides of strip. Most test sites. Reliable detection results.</td>
<td>Smallest meter. Most sites available. Low potential measurement. Reliable detection results.</td>
<td>7, 14 or 30 days average available. Audible test reminder.</td>
</tr>
</tbody>
</table>
Error grid analysis categorizes individual reported blood glucose value by probable clinical consequences of inaccuracy. Figure 2-1 is an example of error grid analysis[13, 14]. In zone A, the values lie within ±20% to the reference, and are considered clinically accurate. Zone B is where the error is greater than ±20%, but would be benign and no treatment action should be taken. In zone C, unnecessary correction or overcorrection of blood glucose could be given. In zone D, hypo- or hyperglycemia is failed to be detected, leading to a dangerous failure to treat. In zone E, the reported and reference values differ substantially, which leads to erroneous treatment of hypo- or hyperglycemia. The estimates within zone A and B are clinically acceptable, but those within zone C through E are potentially dangerous.

![Error Grid Analysis](image)

Figure 2-1: Error Grid Analysis.(From: D. A. Gough and E. L. Botvinick, 1997)

Blood glucose monitors have been found to be accurate and reliable if correctly used and most monitors provide results within one minute. Most glucose monitors can accurately
place 95% of samples in zone A in laboratory testing. Studies have shown that 94-100% of readings were clinically accurate, within zone A in an error grid analysis when the meter is in the hands of an experienced pathology nurse[12, 15-17]. However, in real world operation this can fall to 70% of samples within in zone A due to incomplete samples, interstitial fluid mixing with blood and patient errors.

Another statistical method is called Bland-Altman analysis. An example is illustrated in Figure 2-2[18]. It is often used for assessing agreement between two methods of clinical measurement. This method for evaluating glucose meters results in a plot of the mean and the difference from the mean, bounded by two times the standard deviation, as a function of the mean value.

Figure 2-2: Bland-Altman Analysis(From: A. Kerssen et al., 2005)
2.1.4.2 Continuous Glucose Monitoring Systems

The results of multiple self-blood glucose tests per day with intensive insulin therapy showed that patients’ HbA1c levels were reduced clinically and statistically[10, 11]. Nevertheless, intermittent glucose testing can not be performed during sleep or when a patient is occupied, and may miss an episode of hypo- or hyperglycemia. In recent years, researchers have made breakthrough progress for enabling continuous glucose monitoring (CGM). There are a couple of continuous glucose monitors approved by the Food and Drug Administration (FDA) and available on the market. These devices provide almost all the information required for people with diabetes to optimize their insulin therapy and metabolic control. It allows quick treatment to be given and potentially avoids life-threatening hypo- or hyperglycemia events. Ultimately it is hoped that these CGMS could be linked to an insulin pump to provide feedback controlled insulin delivery to a diabetic patient. It has to be noted that all the continuous monitoring systems sample the glucose concentration in interstitial fluid and there may be a physiological lag time between changing blood glucose levels and interstitial glucose levels which can not be overcome by improvements in technology.

GlucoWatch Biographer

This system is the first CGMS approved by FDA, which utilizes reverse iontophoresis to draw interstitial fluid containing glucose from skin into hydrogel pads of a glucose oxidase biosensor. The GlucoWatch appears like a wearable watch on the wrist displaying the time and glucose levels (Figure 2-3 from GlucoWatch website).
Since the device is non-invasive, it is considered a good continuous glucose monitoring option especially for children with diabetes. The system requires a 2 hour warm-up period and provides automatic noninvasive readings as frequently as every 10 minutes for up to 13 hours. At the beginning of every 13-hour session, a single calibration is required. The manufacturer claims 95% of readings are clinically accurate and acceptable, and the system is generally in good agreement with reference blood glucose reading. However, studies have found that the system does not detect all episodes of hypoglycemia efficiently and reliably as shown in Figure 2-4[19]. Other common problems with this device have been skin irritation and skipped readings due to sweating, cold temperature and activity[20, 21]. These problems led the FDA to approve the Glucomon as a supplemental device which means that any hypo- or hyperglycemia reading must be confirmed by a standard finger prick glucose monitor. This has made it difficult for patients to have the Glucomon covered by insurance and has limited its penetration into the market place.
Figure 2-4: Representative GlucoWatch® biographer results compared to blood glucose measurements for the clinical setting trial.
Medtronic MiniMed CGMS Guardian

The Guardian System consists of a tiny subcutaneously implanted enzyme sensor, a transmitter, and a monitor, about the size of a cell phone, which displays real-time glucose readings (Figure 2-5 from Medtronic website). It is based on the same technology as the Minimed CGMS Gold. The main difference is that the Guardian system provides patients with ongoing and continuous glucose monitoring while the CGMS Gold was developed as a three-day diagnostic tool, specifically for physician use. Both systems provide an average blood glucose measurement every five minutes, 288 readings a day, for up to 72 hours. After 72 hours, the sensor is replaced and the data can be downloaded using proprietary software and a docking system for further analysis. The system also requires calibrations by obtaining blood glucose values from a standard finger-prick sample every 12 hours and before insulin therapy is adjusted.

Figure 2-5: The Medtronic MiniMed CGMS Guardian
Studies showed that the CGMS system is a useful tool for glycemic control since it provides patients and physicians with useful and abundant data and detects more episodes of hypo- and hyperglycemia than intermittent SBGM[18, 22, 23] as shown in Figure 2-6[21]. The system, however, still struggles for accuracy at low glucose concentrations. Underestimating and overtreating hypoglycemia have both been observed[14, 24]. It is suggested to be used only as an auxiliary method of daily glucose level measurement as a result of the small degree of error.

GlucoDay Microdialysis System

The GlucoDay is based on microdialysis for continuous glucose sampling from interstitial fluid. A semipermeable dialysis fiber is inserted subcutaneously into the abdominal wall and perfused with glucose-free isotonic fluid. Glucose from tissue diffuses into the fiber and is pumped to a glucose sensor outside the body where the glucose concentration is measured continuously (Figure 2-7 from Menarini website).
The system provides a reading every three minutes for 48 hours. Only one calibration is needed for the 48 hours. The in vivo continuous glucose monitoring using the system for 24 hours in a type 1 diabetic is shown Figure 2-8[25]. It has been reported to have better accuracy, precision and long-term stability since foreign body reactions are avoided[21, 26-28]. A disadvantage is the instrumental time lag inherent to the microdialysis technique, estimated to be about 7 minutes[28].
Figure 2-8: In vivo glucose sensing using the GlucoDay in a type 1 diabetic for 24 hours, compared to intermittent blood glucose self-testing with capillary blood samples.
2.2 Microdialysis

Microdialysis is a continuous sampling technique based on controlling the mass transfer rate of small molecules across a semipermeable membrane while excluding the larger ones. For biochemical monitoring, microdialysis systems are usually placed (inserted or implanted) inside the tissue of interest with an isotonic perfusion fluid flowing through the system and diffusional exchange occurring between the perfusate and the surrounding interstitial fluid (ISF). Since the dialysis process does not change or affect the surrounding fluid, it is viewed as a tool for continuous monitoring. Microdialysis systems have recently been adapted to microfluidic technologies for sample preparation prior to sample analysis or for miniaturized biochemical probes.

2.2.1 Development of Microdialysis

Researchers have been interested in the biochemical functions of the body in vivo, especially in the extracellular compartment where signaling chemicals can affect cellular function. Neuroscientists were the pioneers to develop microdialysis techniques due to their interest in analyzing neuron signaling processes within intact brains. The technique was originally used in neuroscience for the measurement of neurotransmitters. It allows sampling of brain regions in conscious, freely moving animals and directly delivering drugs into specific areas. There have been experimental attempts to analyze the tissue of interest without changing or affecting the surrounding fluid environment. The concept of introducing a semipermeable membrane into the tissue of interest to dialyze or continuously sample molecules was first introduced in the late 1960’s and early 1970’s.
In 1966, Bito et al. implanted membrane-lined “dialysis sacs” containing 6% dextran in saline solution into the cerebral hemispheres of dogs. The sacs were removed surgically after ten weeks and analyzed for the content of amino acids. This experiment introduced the idea of using membrane dialysis compartment to monitor the extracellular environment[29, 30].

In 1972, Delgado et al. developed the first primitive version of the present microdialysis probes, called “dialytrode”[31]. The dialytrode consisted of two stainless steel tubes soldered together forming a push-pull cannula ending with a small permeable bag. They were the first to perfuse the dialysis membrane in vivo in monkeys and the first to practice the technique which has since become known as “microdialysis”[32].

Development of microdialysis from a long-term dialysis sac implantation, to the push-pull cannula, and finally to the current continuous perfusion flow sampling/monitoring emerged in the neuroscience field in 1974 when Ungerstedt and Pycock first developed a linear, hollow-fiber probe design to continuously monitor neurotransmitter concentrations within rat brains[30, 33]. They implanted the probe by drilling holes in each temporal bone of rats and guided the fiber into an area of the brain known to be devoid of a blood brain barrier. Since then, more designs of microdialysis probes have been presented and utilized not only in neuroscience but also in other fields when continuous sampling/monitoring is needed. Today, the technique is widely applied to almost all body organs and fluid, including blood, heart, liver, kidney, bile, lung, bone, eye, muscle, and breast. Microdialysis has been used in the medical community to continuously monitor various metabolites in patients.

Aside from monitoring biomolecules in humans and animals, the microdialysis technique is also utilized in the area of environmental research. The probes are inserted into
freshwater systems or soil for real-time and continuous sampling with minimum disturbance of the outer environment[34].

2.2.2 Basic Principles

The most common type of microdialysis probe is constructed as a concentric tube as shown in Figure 2-9. The probes usually consist of a semi-permeable membrane, such as polysulfone, polyethersulfone, polyamide, polycarbonate-polyether copolymer and cuprophan[35], glued between the tip of the inner cannula and the outer shaft, which are made of steel or plastic. The perfusion fluid (perfusate) enters the inlet flowing through the inner tube to its distal end and exits the inner tube to enter the space between the inner tube and the outer dialysis membrane where molecular exchange takes place. After the exchange, the fluid containing the molecules of interest (dialysate) is transferred toward the proximal end of the probe and is collected at the outlet for later analysis. Several different types of microdialysis probe construction are commercially available, including linear, loop, concentric and side-by-side as shown in Figure 2-10[36].
Several types of commonly used CMA microdialysis probes (From CMA microdialysis website) are shown in Figure 2-11.
In order to conduct microdialysis experiments several other components are required. Syringe pumps are often used to control the perfusate flow rate. The pump has to be able to deliver flow rates precisely in the µL/min range. Tubing is needed to connect between the probe and the pump which drives the perfusion flow and in some cases, between the probe and a sample collector as well. The total dead volume of tubing should also be maintained as small as possible to have better time resolution. The perfusion fluid is a medium resembling the composition of extracellular fluid with minimal or zero concentration of the interested molecules. Dialysate exiting from the outlet of the microdialysis probe is usually collected in a vial for later analysis. It is also possible to connect the outlet directly to an analysis instrument without using a collector, which is usually preferred, if possible, for its convenience and faster analysis results.

Figure 2-11: CMA Microdialysis Probes

- **CMA/11 Microdialysis Probe**
  - O.D of steel shaft: 0.38mm
  - Cuprophan membrane with O.D. of 0.24mm.
  - Cutoff: 6000 Daltons

- **CMA/7 Microdialysis Probe**
  - O.D of the probe 0.24mm, extremely small and optimized for CNS use.
  - Cuprophan membrane
  - Cutoff: 6000 Daltons

- **CMA/20 Microdialysis Probe**
  - Soft, non-metallic construction and ideal for metabolism studies.
  - Polycarbonate membrane or polyethersulfone membrane
  - Cutoff: 20000 and 100000 Daltons

- **CMA/7 Microdialysis Probe**
  - O.D of the probe 0.24mm, extremely small and optimized for CNS use.
  - Cuprophan membrane
  - Cutoff: 6000 Daltons
The concentration gradient between the perfusate and the component of interest in the ISF (e.g., glucose) is the driving force to transport molecules to the lumen of the probe. The concentration of the molecules of interest in the perfusion fluid at the output of the microdialysis probe is a representative of the ISF and can be correlated to the concentration within the ISF. The term, recovery, is defined as

\[
\text{Recovery} = \frac{C_{d,\text{out}} - C_{d,\text{in}}}{C_r - C_{d,\text{in}}} \quad \text{Equation 2.1}
\]

where \(C_{d,\text{in}}\), \(C_{d,\text{out}}\), and \(C_r\) are the concentration of the interested analyte in the inflow perfusate, outflow dialysate and the exterior surroundings. When \(C_{d,\text{in}}\) equals zero, the recovery can be simplified as

\[
\text{Recovery} = \frac{C_{\text{out}}}{C_\infty} \quad \text{Equation 2.2}
\]

where \(C_{\text{out}}\) equals \(C_{d,\text{out}}\) the outlet concentration and \(C_\infty\) equals \(C_r\) the bulk concentration in the surrounding fluid.

### 2.2.3 Quantitative Microdialysis Models

Microdialysis recovery, also known as “dialysate extraction fraction”, is described as

\[
\text{Recovery} = E_d = \frac{C_{d,\text{out}} - C_{d,\text{in}}}{C_r - C_{d,\text{in}}} = 1 - \exp\left[\frac{-1}{Q_d (R_d + R_m + R_{\text{ext}})}\right] \quad \text{Equation 2.3}
\]

where \(C_{d,\text{in}}\), \(C_{d,\text{out}}\), and \(C_r\) are the concentration of the interested analyte in the inflow perfusate, outflow dialysate and the exterior surroundings and \(Q_d\) is the perfusate/dialysate flow rate. The equation was proposed and demonstrated by Bungay and his co-workers with the assumptions that the membrane hydraulic conductivity and transmembrane pressure
differences are sufficiently small and no appreciable transmembrane fluid flow was produced [37, 38].

The overall probe-surroundings permeability (K) and surface area (A) product is

\[ KA = \frac{1}{R_d + R_m + R_{ext}} \]  \hspace{1cm} \text{Equation 2.4}

Equation 2.4 is governed by the resistance of analyte transport through three regions: the external surrounding medium (\(R_{\text{ext}}\)), the membrane (\(R_m\)), and the dialysate (\(R_d\)). Since they are in series and the mass transfer flow is the same across the three regions, the three resistance are additive.

A schematic of the cross-section of a microdialysis probe is shown in Figure 2-12, of which \(r_i\) is the inner radius of the inner cannula, \(r_a\) is the outer radius of the inner cannula, \(r_\beta\) is the inner radius of the membrane, and \(r_o\) is the outer radius of membrane.

\[ R_d = \frac{13(r_\beta - r_a)}{70\pi L r_\beta D_d} \]  \hspace{1cm} \text{Equation 2.5}

where \(L\) is the effective dialysis membrane length, and \(D_d\) is the diffusion coefficient though the dialysate.
where $D_m$ is the diffusion coefficient through the membrane and $\phi_m$ is the void volume of the membrane.

It is not possible to generalize $R_{ext}$ based on the knowledge of the molecular weight, shape, charge and probe dimensions. In order to estimate $R_{ext}$, efflux to the microvasculature, metabolism and cell membrane transport process are required. However, as a reference compared with the tissue resistance, $R_q$, resistance of $R_{ext}$ for the probe placed in a quiescent dialysate solution, in which diffusion occurs, can be obtained as

$$R_{ext} = \frac{1}{2\pi D_q \phi_q \sqrt{2r_o L}}$$  \hspace{1cm} \text{Equation 2.7}$$

where $D_q$ is the diffusion coefficient of the surrounding medium, $\phi_q$ is the medium volume fraction accessible to the diffusing species. In addition, $R_{ext}$ is approximately zero, as the medium is well stirred.
From Equation 2.5, Equation 2.6, and Equation 2.7, it is known that the recovery can be optimized by varying the inner cannula geometry even with predetermined inner and outer radii. The effect of the radius of the inner cannula, \( r_s \), were shown to be negligible, which is expected from the equations. On the other hand, the increased outer radius of the inner cannula, \( r_\alpha \), causes a significant increase in recovery, since increased \( r_\alpha \) results in decreasing the resistance of dialysate (\( R_d \)) due to shrinking the diffusional path length (\( r_f r_\alpha \)) [39].

### 2.2.4 Factors Affecting Recovery

In general, it is desirable to operate microdialysis systems at the highest recovery possible. There are several factors which affect the recovery rate of analytes.

#### 2.2.4.1 Perfusate Flow Rate

Because recovery is determined by a competition between a diffusional and perfusion convective flux, it is perfusate flow rate dependent. As demonstrated in Equation 2.3, recovery increases as the flow rate decreases. Even though low flow rates result in high recovery, it is often restricted by the reproducibility of low flow rates supplied by syringe pumps and the sample volume needed for solution analysis. Therefore, extremely low flow rates are often not applicable due to limited analysis time resolution because of the long times needed to collect the appropriate solution volume for analysis.
2.2.4.2 Dialysis Membrane Area and Membrane Material

The dialysis membrane chosen plays an important role in controlling the recovery. The dialysis area of the membrane can be increased to achieve high recovery without sacrificing time resolution. A larger diffusional area can enhance recovery, but in most cases, a minimal tissue disturbance by small microdialysis probes is favored. Recovery can be improved by selecting membranes with a higher permeability, $K$. Overall permeability mainly depends on the properties of the microdialysis membrane, such as pore size, pore density, tortuosity, and analyte particle size. Membranes are commonly available with different molecular weight cut-offs (MWCO), which determines the size of recovered particles. Molecules beyond the MWCO are hindered from entering the microdialysis pores. Membranes with lower MWCOs usually have lower permeability to all species due to a smaller pore size. Recovery of certain analytes can also vary by as much as 20% between dialysis membranes made from different materials with the same MWCO. Several different types of membrane are used: polysulfone, polyethersulfone, polyamide, polycarbonate-polyether copolymer, cuprophan, polycarbonate, regenerated cellulose, cellulose acetate and polyacrylonitrile. They come in different thicknesses, where a thicker membrane decreases the permeability and hence recovery.

2.2.4.3 Diffusion Coefficient

Another parameter which affects recovery is the diffusion coefficient of the molecules of interest, which can be estimated by the Stokes-Einstein equation:
where $k_b$ is the Boltzmann constant, $T$ is the absolute temperature ($K$), $\eta$ is the viscosity of the solvent and $r$ is the particle radius. The diffusion coefficient of molecules is approximately inversely proportional to its radius and hence to the cube root of its molecular weight. This relationship can be described as

$$D = \frac{k_b T}{6\pi \eta r^3}$$  \hspace{1cm} \text{Equation 2.8}$$

Therefore most microdialysis membranes will allow the rapid passage of small molecules but usually struggle for higher recoveries of larger molecules due to their smaller diffusivity. It is also obvious that the diffusion coefficient is directly proportional to the temperature. Empirically, the diffusion coefficient for small molecules increases 1-2% per degree centigrade. Thus, it is crucial to carry out the entire microdialysis experiment at a constant temperature.

### 2.2.5 Calibration Techniques

As the relative recovery will never reach 100%, the dialysate concentrations are only a fraction of the true concentration of the interested surrounding fluid. Before using a microdialysis probe for continuous sampling or monitoring, the true concentration of the interested analyte in the surrounding environment and the recoveries at certain perfusate flow rate have to be obtained. There are many different methods for calibration, which are discussed as follows.


2.2.5.1 Perfusate Flow Rate Variation

Calibration is accomplished by varying the perfusate flow rate. At low flow rates, the dialysate concentration reaches a plateau, which is assumed to be near 100% recovery[40-42]. Recoveries at different perfusate flow rates can then be calculated. The possible disadvantages are long sampling time at low flow rates, and only an estimate of the actual value obtained[33].

2.2.5.2 No-net Flux Method

This is a method enabling an estimate of the in vivo recovery introduced by Lonnroth in 1987[43]. The procedures involve perfusing fluid of different concentrations and measuring the difference between perfusate and dialysate concentration. The left hand recovery of Equation 2.3 can be assumed to be an unknown value \( P \) since the perfusate flow rate and resistance are left unchanged. It is rearranged as

\[
C_{d,\text{out}} - C_{d,\text{in}} = -P(C_{d,\text{in}} - C_r)
\]

Equation 2.10

By varying the concentration of \( C_{d,\text{in}} \) and collecting \( C_{d,\text{out}} \) values, a plot in the form of Figure 2-13 is obtained. The intercept with the x-axis is the actual \( C_r \) and the slope reflects the recovery at the selected perfusate flow rate[33, 44].
2.2.5.3 Retrodialysis

This technique is less time-consuming compared with previous ones and the recoveries can be measured continuously during the experiment. It is also called delivery method[45, 46], and is operated by using a perfusate spiked with the analyte of a known concentration. Hence, instead of collecting the analyte from the surrounding tissue, it is delivered into the tissue. By neglecting $C_r$ under the assumption of it being negligible when compared with the known $C_{d,in}$, the recovery is determined as

\[
\text{Recovery} = 1 - \frac{C_{d,out}}{C_{d,in}} \quad \text{Equation 2.11}
\]

It is recommended that the perfusate concentration to be at least ten times the concentration of the periprobe fluid[33].

Another variation of this method is adding a marker or internal standard to the perfusate[47]. The marker should match the diffusion characteristics of the analyte and
therefore the recovery can be obtained in vivo by measuring its relative loss through the microdialysis process (Equation 2.11)[48].

2.2.6 Microdialysis for the Management of Diabetes Mellitus

In diabetics, the consistently high glucose levels result in long-term complications including retinopathy, nephropathy and neuropathy which often leads to amputation of extremities. Studies have shown that tight glycemic control reduced the complications dramatically[10, 11]. However, one of the disadvantages of the glycemic control was a tripling of the risk of hypoglycemic incidences highlighting the need for a feedback controlled insulin infusion system or ‘artificial pancreas’. One of the critical components of such a system is a continuous microdialysis based glucose sensing system[49-51].

By controlling the mass transfer rate of molecules of different sizes across a semipermeable membrane, microdialysis is considered to be a continuous sampling technique. The commercially available probes are inserted into the tissue of interest directly. In diabetes treatment, these probes are usually inserted subcutaneously into either the abdomen or forearm sampling glucose from the interstitial space[52-58].

The probes have been used for diabetes treatment as a continuous sampling tool. The dialysate exiting from the probe is then transferred to a glucose sensor by a tubing connection. This set-up struggles to obtain high recoveries of analytes and has lengthy monitoring lag caused by a large dead volume from the extended tubing within the system. A wide range of delay times have been reported, from 2 min up to 40 min[53, 57, 59]. Some studies proposed integrated systems of the microdialysis probes and a biosensor while the
sampling probes and glucose sensors are still fabricated as separate pieces and then assembled together[25, 27, 54, 58, 60-66].

2.2.7 New Frontier – Microdialysis Chips

For the past ten to fifteen years, semipermeable membranes have been utilized in microfluidics[67]. The concept of a micro total analysis system (µTAS) was introduced by Widmer and colleagues in 1990, in which silicon chip analyzers incorporate sample pretreatment, separation, and detection[68]. The advantages of microchip analysis include the ability to analyze minute samples, speed of analysis, reduced cost and waste, and portability. Ever since, the µTAS has drawn a huge amount of attention and researchers have been attempting to incorporate a microdialysis based continuous sampling component. Some of the devices which have been fabricated are shown in Figure 2-14.

One approach towards integrating microdialysis membranes with microfluidic systems has been to sandwich a commercially available dialysis membrane between two microfluidic chips. Pan and colleagues adopted this approach for continuous glucose monitoring[69]. In their experiment a commercially available microdialysis probe was used to continuously collect glucose from an analyte solution and the dialysate from the microdialysis probe was introduced into an analysis module consisting of two sets of microfluidic channels with a patterned interdigitated electrode array separated by a microdialysis membrane (Figure 2-14 a). The dialysate containing the glucose is introduced into one set of channels and allowed to diffuse through the microdialysis membrane into the other set of channels containing glucose oxidase (GOx) enzyme which oxidizes the glucose
into gluconic acid and hydrogen peroxide. The hydrogen peroxide is subsequently oxidized on the electrode surface to produce a working current which is proportional to the glucose concentration in the dialysate. The microdialysis membrane was sandwiched between the two sets of channels to prevent the GOx from diffusing into the glucose dialysate and being depleted from the system and thus improves sensor lifetime. In this work the layers are held together by mechanical pressure.

A second approach by Kirby and colleagues[70] to integrating microdialysis membranes with microfluidic channels used a UV laser photopolymerization technique to

Figure 2-14: Microdialysis Chips
lithographically define a patterned nanoporous dialysis membrane within a microfluidic channel (Figure 2-14 b). A prepolymer solution is allowed to flow through the microfluidic structure and patterned by phase separation photopolymerization using a shaped UV laser beam. The microdialysis membrane was defined along a series of posts which separated to compartments within a microfluidic network. By changing polymer precursors, the degree of crosslinking could also be adjusted to affect the membrane MWCO. Countercurrent flow operation was demonstrated. The first dialysis demonstration used a membrane with a MWCO below 5700 which was designed for desalting of protein solutions. A low molecular weight rhodamine dye was observed to freely penetrate across the membrane which a higher molecular weight (MW 5,700) insulin protein could not. Next, a higher MWCO membrane was defined for fractionation of a mixed protein solution. Using, this membrane lactalbumin (MW 14,000) was seen to permeate across the membrane.

A final approach to integrating microdialysis membranes with microfluidics is based on direct bonding of microdialysis membranes with the microchannel structures. One such structure was developed by Bohn and colleagues[71] and is shown in Figure 2-14 c. Here microchannels are defined in polydimethylsiloxane (PDMS) in the soft lithography process, activated in an oxygen plasma and directly bonded to a nanoporous track-etched polycarbonate membrane. Bohn and colleagues used this device for electrically controlling sample manipulation after capillary electrophoresis (CE) separation of arginine and glutamate. After they were separated, either the arginine or glutamate was collected through the microdialysis membrane into a different microfluidic channel by controlling the time at which the gate was biased to collect the sample flowing through that area of the CE column.
In this thesis, a novel on-chip microdialysis system which addresses some of the problems with continuous glucose monitoring is presented[72-74]. The device is based on thin-film fabrication, which is easily integrated with in-situ biosensors, and direct polymer bonding on microfluidic channels designed for direct contact between dialysis membrane and tissue of interest. The integration of a biosensor directly with the microdialysis system is designed to allow high recovery of analytes with a smaller diffusional surface area and lower flow rates resulting in a less invasive, more precise microdialysis probe. In addition, the large surface to microchannel volume ratio and short diffusional path will allow higher recoveries and faster equilibration times for higher frequency sampling rates.
Chapter 3

On-chip Microdialysis System with Impedance Sensing Capability

As a first step towards the integration of a microdialysis system and a biosensor, continuous sensing is demonstrated by measuring changes in solution conductivity in a dialyzed solution. In this chapter, an on-chip microdialysis system with gold sensing electrodes for ion impedance sensing is developed.

The device is based on thin-film fabrication, which is easily integrated with in-situ biosensors, and direct polymer bonding on microfluidic channels designed for direct contact between dialysis membrane and tissue of interest. In order to create a reservoir environment with the concentration continuously being changed for testing the time resolution of the device, a stacked system and a micro-mixer were designed to cooperate with the microdialysis chip.

The integration of a biosensor directly with the microdialysis system is designed to allow high recovery of analytes with a smaller diffusional surface area and lower flow rates resulting in a less invasive, more precise microdialysis probe.

3.1 Theory

Analytical mass transport modeling for the microdialysis system is induced here. The concept of electrolytic conductivity of solution is explained and provides the basis to determine the concentration/recovery of the dialysate. A micro-mixer was used to produce
well-mixed solution of known concentration being sent to the stacked system. Section 3.1.4 illustrates the strategy of designing the micro-mixer.

### 3.1.1 Thin Film Model

A thin film model is used here to predict the mass transfer across a dialysis membrane[75]. This approach assumes that all of the resistance to mass transfer in the following fluids is localized in stagnant films of thickness $\delta_B$, $\delta_M$ and $\delta_D$ (for tissue, membrane and dialysate respectively). The model is simply described by an “equivalent” two zone model of stagnant and fully mixed regions as in Figure 3-1.

---

Figure 3-1: Thin-film model
Fick’s laws of diffusion are used as a starting point. It is assumed that the same partition coefficient, $\alpha$, can be used on both sides of the membrane.

$$\alpha = \frac{C'M}{C'B} = \frac{C''M}{C''D}$$  \hspace{1cm} \text{Equation 3.1}

In this work, it is assumed that the partition coefficient, $\alpha$, equals 1.

At steady state, the fluxes crossing through both fluid films and through the membrane are all equal, so that

$$N = \frac{D_B (C_B - C'B)}{\delta_B} = \frac{D_D (C''D - C_D)}{\delta_D} = \frac{D_M (C''M - C'M)}{\delta_M}$$  \hspace{1cm} \text{Equation 3.2}

Where $D_B$, $D_D$ and $D_M$ stands for the diffusion coefficients of the two fluid films and membrane.

The overall concentration difference is

$$C_B - C_D = (C_B - C'B) + (C'B - C''D) + (C''D - C_D)$$  \hspace{1cm} \text{Equation 3.3}

Using the partition coefficient, the flux is

$$N = \frac{D_M \alpha (C'M - C''M)}{\delta_M \alpha} = \frac{D_M \alpha (C'B - C''D)}{\delta_M}$$  \hspace{1cm} \text{Equation 3.4}

From Equation 3.2, it is also evident that

$$C_B - C' = \frac{\delta_B N}{D_B}$$  \hspace{1cm} \text{Equation 3.5}

and

$$C''D - C_D = \frac{\delta_D N}{D_D}$$  \hspace{1cm} \text{Equation 3.6}

Hence
Or

\[ N = K_o (C_B - C_D) \]  

Equation 3.8

Where \( K_o \) is the overall mass transfer coefficient and \( 1/ K_o \) is the overall resistance given as

\[
\frac{1}{K_o} = R = R_B + R_M + R_D = \frac{\delta_M}{D_B} + \frac{\delta_M}{\alpha D_M} + \frac{\delta_D}{D_D} = \frac{1}{K_B} + \frac{1}{\alpha K_M} + \frac{1}{K_D} \]

Equation 3.9

3.1.2 Mass Transfer Prediction

For this microdialysis system, molecules inside the reservoir are dialyzed across a membrane. Assuming the mass transfer coefficient is kept constant, as \( K_o \), over the diffusion path.

---

**Figure 3-2**: Schematic of the Mass Transfer of the On-chip Microdialysis System

The mass flux crossing the membrane is given by
\[ \frac{d\hat{m}}{dt} = -Q_r \frac{dC_r}{Q_r} \]  

Equation 3.10

where \( Q_r \) is the flow rate of the reservoir flow channel, and \( C_r \) is the solute concentration. By rearranging Equation 3.11,

\[ dC_r = -\frac{d\hat{m}}{Q_r} \]  

Equation 3.11

The convective flux of the perfusion fluid is given as

\[ d\hat{m} = Q_d dC_d \]  

Equation 3.12

and

\[ dC_d = \frac{d\hat{m}}{Q_d} \]  

Equation 3.13

where \( Q_d \) is the flow rate of perfusion flow channel and \( C_d \) is the solute concentration.

The diffusional mass transfer across the membrane can also be represented as

\[ d\hat{m} = K_0 (C_r - C_d) dA \]  

Equation 3.14

where \( A \) is the diffusional area. By subtracting Equation 3.13 from Equation 3.11,

\[ dC_r - dC_d = -\frac{d\hat{m}}{Q_r} - \frac{d\hat{m}}{Q_d} = -d\hat{m} \left( \frac{1}{Q_r} + \frac{1}{Q_d} \right) \]  

Equation 3.15

By substituting Equation 3.14 into Equation 3.15,

\[ d(C_r - C_d) = -K_0 (C_r - C_d) \left( \frac{1}{Q_r} + \frac{1}{Q_d} \right) dA \]  

Equation 3.16

Equation 3.16 can be rearranged into

\[ \frac{d(C_r - C_d)}{(C_r - C_d)} = -K_0 \left( \frac{1}{Q_r} + \frac{1}{Q_d} \right) dA \]  

Equation 3.17

By integrating Equation 3.17 along the membrane diffusional area, \( A_m \)
\[
\ln \frac{C_{r, \text{out}} - C_{d, \text{out}}}{C_{r, \text{in}} - C_{d, \text{in}}} = -K_0 A_m \left( \frac{1}{Q_r} + \frac{1}{Q_d} \right)
\]

Equation 3.18

\(C_{r, \text{in}}\) and \(C_{r, \text{out}}\) are the inlet and the outlet concentration of PDMS reservoir channel. \(C_{d, \text{in}}\) and \(C_{d, \text{out}}\) are the dialysate inlet and outlet concentration of the perfusion flow. \(Q_r\) is the PDMS reservoir channel flow rate and the \(Q_d\) is the perfusion flow rate. \(K_0\) is the overall molecular permeability and \(A\) is the diffusional surface area.

In this work, \(C_{d, \text{in}}\) is always zero. Since the reservoir flow rate is much higher than the perfusion flow rate, \(C_{r, \text{in}}\) and \(C_{r, \text{out}}\) are assumed to be equal values, \(C_\infty\), and \(C_{d, \text{out}}\) is simply \(C_{\text{out}}\). In addition, when \(Q_r\) is much larger than \(Q_d\) \((1/Q_r << 1/Q_d)\), the \(1/Q_r\) can be ignored on the left of the equation. Therefore, Equation 3.18 can be simplified as

\[
\ln \left(1 - \frac{C_{\text{out}}}{C_\infty}\right) = -K_0 A_m \left( \frac{1}{Q} \right)
\]

Equation 3.19

where \(Q\) stands for \(Q_d\). Eqn. 3 can be rearranged to represent recovery as a function of perfusion flow rate.

\[
\frac{C_{\text{out}}}{C_\infty} = 1 - e^{-\frac{K_0 A_m}{Q}}
\]

Equation 3.20

Also by plotting the left side of Equation 3.19, \(-\ln(1-C_{\text{out}}/C_\infty)\), as a function of perfusion flow rate \((1/Q)\), the permeability of the membrane to the molecules of interest (the slope as \(K_0 A\)) may be determined to characterize the system functionality.
3.1.3 The Concept of Electrolytic Conductivity

An electrolyte is a chemical compound that dissociates into free ions in solid or liquid and behaves as an electrically conductive medium. The ability of an electrolyte solution to sustain a passage of electrical current depends on the mobility \( u \) of its free ions in the electric field between two electrodes immersed in the solution.

The velocity \( v \) of ions of charge \( z e_0 \) in the solution is subject to the electric field strength, \( E \), and also to the frictional force, \( K_R \). For simple spherical ions of radius \( r_i \), the frictional force, \( K_{R_i} \), is given by Stokes formula as

\[
K_{R_i} = 6\pi \eta r_i v
\]

where \( \eta \) is the viscosity of the medium.

The result of the electrical and frictional forces attains a limiting velocity, \( v_{\text{max}} \):

\[
ze_0 E = 6\pi \eta r_i v_{\text{max}}
\]

and the terminal velocity is given by

\[
v_{\text{max}} = \frac{z e_0 E}{6\pi \eta r_i}
\]

The amount of charge carried through the surface per unit time, or the current per area \( A \), is given by

\[
I = I^+ + I^- = \frac{dQ^+}{dt} + \frac{dQ^-}{dt} = A e_0 (n^+ z^+ v_{\text{max}}^+ + n^- z^- v_{\text{max}}^-)
\]

The summation in Equation 3.24 can be extended if there are more than two types of ions present in the solution.

The mobility, \( u \), is defined as:

\[
u = \frac{dQ}{dt}
\]
which is a scalar quantity. By substituting Equation 3.25 in Equation 3.24,

\[ I = A e_0 (n^+ z^+ u^+ + n^- z^- u^-) \cdot |E| \quad \text{Equation 3.26} \]

If the applied potential is \( \Delta V \) between the electrodes which are separated by a distance, \( l \), the magnitude of the electrode field is

\[ |E| = \frac{\Delta V}{l} \quad \text{Equation 3.27} \]

then the current becomes

\[ I = A e_0 (n^+ z^+ u^+ + n^- z^- u^-) \frac{\Delta V}{l} = G \Delta V \quad \text{Equation 3.28} \]

where \( G \) is the conductance. So that

\[ G = \left( \frac{A}{l} \right) e_0 (n^+ z^+ u^+ + n^- z^- u^-) \quad \text{Equation 3.29} \]

For strong electrolytes that are dissociated completely in solution, a linear relationship between the conductance and the ion concentration is expected. However, this linear dependence is only approximately true for dilute solutions. At higher concentrations, the conductance rises less rapidly than expected due to the increasing interionic interactions as the mean distance between ions decreases due to the electrostatic forces between oppositely charged ions which hinder the mobility[76].
3.1.4 Micro-mixing

In order to create an environment for testing the time resolution of the device’s continuous monitoring ability, it is necessary to control the concentration of the solution which is sent into the reservoir channel of the stacked system. The concentration is controlled by continuously changing the relative flow rate of two syringe pumps, one is filled with a PBS solution; the other is filled with DI water.

Before the solution is sent into the stacked system, the fluid has to be well-mixed. In the macroscopic world, mixing can be achieved easily and rapidly by mechanical agitation or stirring to cause vortical flows. However, fluid flow in microfluidic devices are usually in the laminar regime with Reynolds numbers on the order of 10 or less, and vertical flow fields are difficult to produce. As a result, the mixing rate is controlled by the rate of diffusion.

The goal here is rapid mixing between the initially segregated flow streams, of PBS solution and of DI water. The average diffusion time $\tau$ over the mixing length $d$ is given by

$$\tau = \frac{d^2}{2D}$$

Equation 3.30

where $D$ is the diffusion coefficient. Equation 3.30 clearly indicates that rapid mixed can be achieved by decreasing the mixing length, which implies a smaller width of the segregated flow streams[77].

The strategy is to split the flow stream into $n$ sub-streams within the appropriately designed microfluidic channel and rejoining them to yield a shorter diffusion pathway, as shown in Figure 3-3. The total mixing time is then decreased by a factor of $n^2$. 

The sufficient channel length \((L)\) for mixing is therefore calculated by

\[
u = \frac{Q_{PBS} + Q_{DI\text{water}}}{A_{\text{cross}}}
\]

Equation 3.31

and

\[
L = u \times \tau
\]

Equation 3.32

where \(Q_{PBS}\) and \(Q_{DI\text{water}}\) are the flow rates of PBS solution and DI water, \(A_{\text{cross}}\) is the cross section area of the channel and \(u\) is the average velocity of the flow.
3.2 Device Fabrication

There are three main parts of the described device: the microdialysis monitoring chip, a PDMS reservoir channel piece and a PDMS mixer. The system is fabricated using a polycarbonate track-etch membrane (100 or 15 nm diameter pore sizes) directly bonded onto SU-8 microfluidic channels with the gold resistance sensing electrodes patterned and sputtered in the bottom of the microchannels\cite{78}(Figure 3-4). A complete fabrication protocol for this device was developed with a non-leaking and reproducible bond between the SU-8 layer and the membrane\cite{72, 73}.

![Figure 3-4: Schematic of the stacked microdialysis system with impedance sensing electrodes](image)

3.2.1 Microdialysis Monitoring Chip

The gold sensing electrodes were first fabricated using lift-off techniques on clean glass slides. The mask of the electrodes is shown in Figure 3-5. The dimensions of the electrodes are 200 \( \mu \text{m} \) x 2000 \( \mu \text{m} \) and separated by 40 \( \mu \text{m} \). The contact pads are 2 mm x 2 mm, which was designed to be large enough for easy wire connection. The redundancy of
two sets of impedance electrodes and contact pads is in case a set electrodes did not function properly, the other set can still be used. This is a dark field mask. The positive photoresist of the white electrodes areas exposed to UV light will be washed away during the development.

---

**Figure 3-5:** Mask for impedance sensing electrodes

Shipley 1818 photoresist was used to define the electrode areas. The detailed photolithography procedure is described in Table 3-1. After the photolithography procedures, the glass slides were cleaned by dipping in 10% hydrofluoric acid solution for 5 seconds and rinsing under running deionized (DI) water. A 3000 Å thick Chrome/Gold layer was then sputtered on the HF-cleaned glass slides. The glass slides were placed in an acetone solution until all the unwanted Cr/Au peeled off. Access holes for the fluidic connection within the device were drilled by estimation before constructing the fluidic channels.
SU-8 2010 negative photoresist is the material chosen for the fluidic channels. The glass slides were again cleaned before applying SU-8. The mask of the fluidic channel is shown in Figure 3-6, in which the width of the channel is 700 µm with perfusion flow inlet and outlet of 1.5 mm x 1.5 mm square. Since SU-8 is a negative photoresist, a bright field mask was used. SU-8 of the dark channel area, not exposed to UV light, is washed away during the development. The microfluidic channel was determined to be 15 µm thick after the standard SU-8 fabrication procedures, as shown in Table 3-2.

Table 3-1: Photolithography procedures for Shipley 1818

<table>
<thead>
<tr>
<th>#</th>
<th>Step</th>
<th>Description</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cleaning Procedures</td>
<td>Soaking glass slides in acetone solution</td>
<td>10 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Soaking glass slides in isopropyl alcohol (IPA) solution</td>
<td>10 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Soaking glass slides in deionized (DI) water</td>
<td>10 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rinse under running DI water</td>
<td>1 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Blow dry with nitrogen gun and place on a hot plate of 200°C</td>
<td>20 min</td>
</tr>
<tr>
<td>2</td>
<td>Spinning Photoresist</td>
<td>Hexamethyldisilazane (HMDS) spinning speed 5500 rpm with a ramp of 1000 rpm/s</td>
<td>5 sec</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Shipley 1818 positive photoresist spinning slow speed 5500 rpm with a ramp of 1000 rpm/s</td>
<td>30 sec</td>
</tr>
<tr>
<td>3</td>
<td>Softbake</td>
<td>Place on a hot plate of 95°C</td>
<td>1.5 min</td>
</tr>
<tr>
<td>4</td>
<td>Exposure</td>
<td>Under the UV lamp of power 10mW/cm²</td>
<td>9 sec</td>
</tr>
<tr>
<td>5</td>
<td>Develop</td>
<td>Soaking in CD-27 solution</td>
<td>1 min</td>
</tr>
<tr>
<td>6</td>
<td>Rinse</td>
<td>Rinse with DI water</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Hardbake</td>
<td>Place on a hot plate of 120°C</td>
<td>15 min</td>
</tr>
</tbody>
</table>
Figure 3-6: Mask for SU-8 microfluidic channel with impedance sensing
Table 3-2: Photolithography procedures for SU-8 2010 of 15 μm depth

<table>
<thead>
<tr>
<th>#</th>
<th>Step</th>
<th>Description</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cleaning Procedures</td>
<td>Soaking glass slides in acetone solution</td>
<td>10 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Soaking glass slides in isopropyl alcohol (IPA) solution</td>
<td>10 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Soaking glass slides in deionized (DI) water</td>
<td>10 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rinse under running DI water</td>
<td>1 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Blow dry with nitrogen gun and place on a hot plate of 200°C</td>
<td>20 min</td>
</tr>
<tr>
<td>2</td>
<td>Spinning Photoresist</td>
<td>SU-8 2010 negative photoresist spinning slow speed 500 rpm with a ramp of 100 rpm/s</td>
<td>10 sec</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SU-8 2010 negative photoresist spinning slow speed 2000 rpm with a ramp of 300 rpm/s</td>
<td>30 sec</td>
</tr>
<tr>
<td>3</td>
<td>Softbake</td>
<td>Place on a hot plate of 55°C</td>
<td>4 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Place on a hot plate of 95°C</td>
<td>4 min</td>
</tr>
<tr>
<td>4</td>
<td>Exposure</td>
<td>Under the UV lamp of power 10mW/cm²</td>
<td>24 sec</td>
</tr>
<tr>
<td>5</td>
<td>Post Exposure Bake</td>
<td>Place on a hot plate of 55°C</td>
<td>4 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ramp the temperature to 95°C</td>
<td>4 min</td>
</tr>
<tr>
<td>6</td>
<td>Develop</td>
<td>Soaking in SU-8 developer</td>
<td>1 min</td>
</tr>
<tr>
<td>7</td>
<td>Rinse</td>
<td>Rinse with IPA</td>
<td></td>
</tr>
</tbody>
</table>

Polycarbonate track-etch membrane (diameter pore size 15 nm, 6 μm thick, Whatman Inc) was chosen as the microdialysis membrane. The membrane and the device were treated with an oxygen plasma for 90 seconds (300 mTorr, 50 W). Before applying the membrane on the SU-8 fluidic channels, the device was wetted with a clean cotton wiper followed by a lamination bond. The device was next placed on a hot plate of 120°C for 30 minutes to enhance the bonding, which subsequently resulted in a strong non-leaking bond (Figure 3-7). The bonding between SU-8 and polycarbonate membrane is also highly
reproducible. The complete microdialysis chip fabrication procedures are shown schematically in Figure 3-8.

Figure 3-7: SEM pictures showing the bonding between the SU-8 layer and the polycarbonate membrane. (Top) a patterned area showing the channel features and bonded membrane. (Bottom) a cleaved slide showing a continuous bond along the length of the field SU-8.
3.2.2 PDMS Pieces

In order to create an environment for testing the device with a changing reservoir concentration more efficiently, a stacked system was designed to fit on the microdialysis chip. In previous studies of glucose microdialysis, a 0.8 mm diameter ring was placed on top of the dialysis channel as a reservoir [72, 79]. The concentration within the reservoir could be changed manually but in a very limited manner. Even though the device was designed for direct contact with a tissue of interest, a second fluidic channel was needed for the time domain testing. Standard soft lithography procedures were employed by using SU-8 2010 for the mold of 20 µm deep reservoir fluidic channels (Table 3-3).
PDMS was poured onto the mold and placed in an oven at 65 °C for 30 minutes for curing. PDMS was chosen to be the material for its ability to also create a non-leaking fluidic channel on the surface of polycarbonate membrane. The PDMS layer was added to imitate the surrounding fluid concentration changes and determine how the microdialysis system

<table>
<thead>
<tr>
<th>#</th>
<th>Step</th>
<th>Description</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cleaning Procedures</td>
<td>Soaking glass slides in acetone solution</td>
<td>10 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Soaking glass slides in isopropyl alcohol (IPA) solution</td>
<td>10 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Soaking glass slides in deionized (DI) water</td>
<td>10 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rinse under running DI water</td>
<td>1 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Blow dry with nitrogen gun and place on a hot plate of 200°C</td>
<td>20 min</td>
</tr>
<tr>
<td>2</td>
<td>Spinning Omnicoat</td>
<td>Omnicoat adhesion promoter spinning slow speed 500 rpm with a ramp of 100 rpm/s</td>
<td>5 sec</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Omnicoat adhesion promoter spinning slow speed 3000 rpm with a ramp of 300 rpm/s</td>
<td>30 sec</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Place on hot plate of 200°C. Wait until the glass slides cool down to room temperature before next step</td>
<td>1 min</td>
</tr>
<tr>
<td>2</td>
<td>Spinning Photoresist</td>
<td>SU-8 2010 negative photoresist spinning slow speed 500 rpm with a ramp of 100 rpm/s</td>
<td>10 sec</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SU-8 2010 negative photoresist spinning slow speed 1000 rpm with 300 rpm/s</td>
<td>30 sec</td>
</tr>
<tr>
<td>3</td>
<td>Softbake</td>
<td>Place on a hot plate of 55°C</td>
<td>7 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Place on a hot plate of 95°C</td>
<td>7 min</td>
</tr>
<tr>
<td>4</td>
<td>Exposure</td>
<td>Under the UV lamp of power 10mW/cm²</td>
<td>28 sec</td>
</tr>
<tr>
<td>5</td>
<td>Post Exposure Bake</td>
<td>Place on a hot plate of 55°C</td>
<td>8 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ramp the temperature to 95°C</td>
<td>15 min</td>
</tr>
<tr>
<td>6</td>
<td>Develop</td>
<td>Soaking in SU-8 developer</td>
<td>4 min</td>
</tr>
<tr>
<td>7</td>
<td>Rinse</td>
<td>Rinse with IPA</td>
<td></td>
</tr>
</tbody>
</table>

Table 3-3: Photolithography procedures for SU-8 2010 of 20 μm depth
responds. The mask of the PDMS reservoir channel is shown in Figure 3-9. The channel width is 1500 µm, much larger than the width of microdialysis channel, which enables one to align two layers by the naked eye. Figure 3-10 shows the alignment of the microdialysis SU-8 channel and the PDMS reservoir channel under microscope.
Instead of placing a ring as a reservoir, a stacked system was developed here to allow time varying reservoir concentrations, as shown in Figure 3-11. The PDMS piece consisted of one inlet and one outlet for infusing solution into the reservoir channel to go through the dialysis area in contact of the semipermeable membrane. PDMS was chosen to be the material for the upper flow channels because of its ability to bond with the semipermeable membrane. PDMS and the membrane surface of the device were both treated with oxygen plasma (300 mTorr, 100 sccm O₂, 50 W) for 1.5 minute and placed in an oven at 65°C for at least 4 hours to cure. The PDMS piece was reinforced by glue along the edge. No leakage was observed during the data collection.
The same soft lithography technique was utilized to build a supportive PDMS micromixer for controlling the input concentration into the reservoir, which was then bonded with a clean glass to close the open surface. Figure 3-12 shows the geometry of the mixer. The width of the mixing channel is 200 \( \mu \text{m} \) and split into 5 sub-streams resulting in a 40\( \mu \text{m} \) diffusional path length and was designed according to the analysis in 3.1.4.

Figure 3-11: Photo of a complete microdialysis system with impedance sensing capabilities

![Image of a complete microdialysis system with impedance sensing capabilities]

Figure 3-12: The geometry of the mixer

![Diagram of the mixer geometry]
3.3 Experimental Setup

Phosphate buffered saline (PBS) solution of varying concentration (1X PBS solution: 10mM phosphate buffer, pH 7.4, 140 mM NaCl, 3 mM KCl) was used to characterize the microdialysis system. If the potential difference between the electrodes is $\Delta V$, and the distance between the electrodes is $l$, then the current flowing between the electrodes is given by Ohm’s law. From Equation 3.28, it can be seen that the solution conductance depends on the nature of the ions dissolved and the concentration of these ions (through $n^+$ and $n^-$).

The experimental setup is shown in Figure 3-13. PE10 tubing (Intramedic polyethylene tubing, I.D.: 0.28 mm, O.D.: 0.61 mm) was used to connect the device to the syringes. Three syringe pumps were employed for time domain microdialysis. Two syringe pumps were required for changing the solution concentration within the reservoir PDMS channel by alternating the relative flow rates from two syringes, where one was filled with 1X PBS solution, the other was filled with DI water. The micromixer was used to generate a well-mixed flow without concentration gradients introduced into the reservoir channel. Splitting the flow stream into n sub-streams and rejoining them will yield a shorter diffusion pathway. The total mixing time is decreased by a factor of $n^2$ (based on $1.3 \times 10^{-9}$ m²/s free diffusion coefficient of Na⁺, and 10 µL/min flow rate). The third syringe pump was used for producing a steady perfusion flow of DI water within the SU-8 lower channel.
An impedance meter (ESI impedance meter 252) was connected to the contact pads of the impedance device and a computer was used to collect the impedance data continuously through a National Instruments digital to analog (D/A) (SCB-68 National Instruments) card. A LabView program (Figure 3-14) was written to control the flow rates of the two alternating syringe pumps continuously and to receive the impedance signal from the D/A card simultaneously.
Figure 3-15: The LabView program to control flow rates of two syringe pumps and to collect impedance data. Notice that the two syringe pump flow rates are 180° out of phase to produce a sinusoidally changing reservoir concentration.
3.4 Results and Discussion

The fabrication protocol results in reproducible and stable devices. During the lift-off procedure for electrodes, the positive photoresist were slightly over exposed to have completely clean electrode pattern surface before depositing Cr/Au on the slides. The metal layer can not be removed by sticking scotch tape onto the gold film, which implies good metal adhesion to the glass slides. The bonding between the SU-8 microfluidic channel and the polycarbonate membrane is also strong and non-leaking. Every device was carefully inspected before and after data collection to confirm that the process of the experiment was not influenced by a leaking membrane, damaged electrodes or some other imperfections.

The diffusion coefficient of sodium ions in bulk water is $1.3 \times 10^{-9} \text{m}^2/\text{s}$. From Equation 3.30, the mixing time was calculated to be 0.615 second. Based on the volume of the mixer and the PDMS reservoir flow rate, it takes 2.52 seconds for fluid to flow through the fluidic channel of the mixer, so the fluid exiting the mixer is considered well mixed.

The relationship between the solution conductance and PBS concentration was first obtained over the experimental operating range. The measured solution conductance as a function of PBS concentration using the microdialysis system with impedance sensing electrodes is shown in Figure 3-16. The calibration is clearly divided into two segments. The slope is larger in the more dilute region, and then decreases as the PBS solution becomes more concentrated, which can be explained by the increasing interionic interactions as the mean distance between ions decreases in the more concentrated PBS solution.
The polycarbonate membrane with 15 nm diameter pore size was tested for PBS microdialysis. PBS recovery as a function of perfusion flow rates was obtained with a device of 700µm x 10mm microdialysis area. The PDMS upper channel was infused with 1X PBS solution at constant flow rate of 10 µL/min and the SU-8 lower channel was infused with DI water at flow rates between 0.1 µL/min to 1.3 µL/min. Outlet solution conductance from the perfusion fluidic channel at different flow rates were obtained and converted to concentration based on the calibration curve. Using Equation 3.19, the permeability of the polycarbonate membrane to ions was determined to be 0.246 µm/s with a standard deviation of 0.028 µm/s as shown in Figure 3-17 and Table 3-4. The recovery at the lowest perfusion flow rate, 0.1 µL/min, is 64.4 % theoretically, which is in good agreement with the experimental results.
Figure 3-17: PBS microdialysis recovery

\[ y = 0.1141193x \]

R-square = 0.99

Experimental Results

Theoretical Results
Experiments with the time varying PBS concentrations within the PDMS reservoir channel were subsequently conducted using both a step change and sinusoidally changing concentration, to evaluate how the device responds to concentration fluctuations within the reservoir fluid. The system response was first demonstrated with step concentration changes in the reservoir at a constant total flow rate of 10 $\mu$L/min. The fluid concentration exiting the PDMS mixer was initially 0.2X PBS, which was changed stepwise to 0.5X PBS. The perfusion flow rate remained constant at 0.1 $\mu$L/min, which results in a 64.4% recovery. The step response of the system was recorded and the system was able to reach a new steady state value after the step concentration change within about 230 seconds (Figure 3-18).

Table 3-4: PBS microdialysis results

<table>
<thead>
<tr>
<th>Experiment</th>
<th>$K_0A_m (\mu$L/min)</th>
<th>$K_0A_m (\mu$m$^3$/s)</th>
<th>$K_0 (\mu$m/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Set 1</td>
<td>0.114</td>
<td>1901988.333</td>
<td>0.272</td>
</tr>
<tr>
<td>Set 2</td>
<td>0.105</td>
<td>1757818.333</td>
<td>0.251</td>
</tr>
<tr>
<td>Set 3</td>
<td>0.091</td>
<td>1510008.333</td>
<td>0.216</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
<td>0.246</td>
</tr>
<tr>
<td>STD</td>
<td></td>
<td></td>
<td>0.028</td>
</tr>
</tbody>
</table>
The system response to any input waveform can be modeled using linear system theory by convolving the input waveform with the impulse response. For a given step response, $g(t)$, the impulse response, $h(t)$, could be obtained by taking the first derivative of the step response.

$$h(t) = g'(t)$$  \hspace{1cm} \text{Equation 3.33}

With the impulse response, the output signal can be predicted.

$$\text{Signal}_{out} = \text{Signal}_{in} \ast h(t)$$  \hspace{1cm} \text{Equation 3.34}

Next, the concentration of the reservoir channel was varied in a sinusoidal fashion from 0.2X PBS to 0.8X PBS by varying the relative flow rate of the two input syringe pumps.

Figure 3-18: Time domain of step PBS dialysis at a perfusion flow rate of 0.1 µL/min
while the total PDMS reservoir flow rate remained at 10 µL/min. The period of the input signal was 800 seconds with a concentration change every 40 seconds. This period was chosen because it is a sufficient time scale for tracking physiological concentration changes such as glucose fluctuations within a diabetic patient. Figure 3-19 shows the sinusoidal response of the system. The input signal is normalized by the recovery of 64.4%. The system was able to reach the predicted recovery with a phase lag of 210 seconds. Both input and output signal were converted into the frequency domain by Fourier transforms and had an obvious frequency peak at 0.00125 Hz, which represents the 800 second sinusoidal period. Matlab was used to process the data.

Figure 3-19: The response of sinusoidal input demonstrated with the impedance microdialysis system
Figure 3-20: Computational simulation using Comsol
A computational simulation of the device has also been conducted using Comsol (Figure 3-20) for the step change and the sinusoidal input. The simulation results showed that the predicted phase lags were about 80 seconds for a step change and 60 seconds for sinusoidal input at the 0.1 µL/min flow rate. The delay due to the dead volume of the mixing device and the tubing connection is therefore estimated to be 150 seconds by dividing the dead volume by the reservoir flow rate, which matches the experimental estimation based on the reservoir flow rate and the tubing dead volume.

A Pearson’s correlation coefficient (r) has been determined between the experimental data and the system response predicted by linear systems theory as well as between the simulation and experimental data. The r-values are 0.98 and 0.97 respectively.

In order to validate the analytical model, the initial assumption that $C_{r,in}$ and $C_{r,out}$ are the same value can be verified by substituting the experimentally determined recovery values and permeability into Equation 3.18. This showed that $C_{r,out}$ and $C_{r,in}$ will differ by less than 1%. Furthermore, the permeability $K_0$ was estimated to be only 3% less when the previously ignored $1/Q_r$ was taken into consideration, which is within the experimental variance measured. Therefore, these assumptions are consistent with the experimental data and the mathematical model presented in Equation 3.19 & Equation 3.20 can represent the microdialysis system well.

Next, mass transport due to pressure difference across the microdialysis membrane and solution osmotic difference are considered. It is assumed that the flow in the channel is incompressible and Newtonian. Hence, the Navier-Stokes equation can be applied here as given by
And the continuity equation is given in

\[ \frac{\partial \rho}{\partial t} + (\nabla \cdot \rho \nu) = 0 \]  

Equation 3.36

The hydraulic model for the rectangular cross section channel can be written as in Equation 3.37.

\[ Q = \frac{4w^3d}{3\mu} \left( -\frac{dp}{dx} \right) \left[ 1 - \frac{192d}{\pi^5w} \sum_{i=1,3,5,...} \frac{\tanh(\pi w/2d)}{i^5} \right] \]  

Equation 3.37

Q is flow rate, w is the half-width of channel, d is the half-depth of channel, \( \mu \) is the water viscosity, p is the pressure[77].

Consequently, the pressure drop along the channel can be obtained by Equation 3.37. The pressure drop along the microchannel can be neglected when compared with the atmospheric pressure. Therefore, the pressure difference across the membrane is assumed to be constant along the diffusional membrane channel.

According to Kedem and Katchalsky’s theory for mass transport across membranes[82], the volume flux, \( J_v \), and solute flux, \( J_s \), are given by

\[ J_v = L_p (\Delta p - \sigma \Delta \Pi) \]  

Equation 3.38

and

\[ J_s = K \Delta C + J_v (1 - \sigma) \bar{C} \]  

Equation 3.39

In Equation 3.38, \( \Delta p \) and \( \Delta \Pi \) are the hydrostatic pressure difference and the osmotic pressure difference of molecules across the membrane. \( \sigma \) is the solute reflection coefficient, which varies from 0 for a freely permeable solute to 1 for an impermeable solute. \( L_p \) is the
hydraulic conductivity, which can be estimated by Curry’s pore theory for flow through a population of uniform cylindrical pores.

\[ L_p = \frac{A_p r_p^2}{8\eta S \Delta x} \]  

Equation 3.40

where \( A_p \) is the total pore area available for transport, \( S \) is the total surface area, \( \eta \) is the solvent viscosity, \( r_p \) is the pore radius, and \( \Delta x \) is the pore length.

The first term of the right hand side of Equation 3.39 represents the diffusive component where \( K \) is the permeability. The second term represents the osmotic pressure-induced flux, also called solvent drag carried across the membrane by the water flux. \( \overline{C} \) is the logarithmic mean of solute concentration on either side of the membrane.

\[ \overline{C} = \frac{(C_1 - C_2)}{\ln(C_1/C_2)} \]  

Equation 3.41

In the microdialysis model presented here, the osmotic pressure-induced flux was found to be negligible when compared with the diffusional flux due to the concentration gradient.

The permeability was obtained as 0.246 \( \mu m/s \) (STD = 0.028 \( \mu m/s \), \( D_m = 1.476 \mu m^2/s \)) and it is in good agreement with the theory. Accordingly, the system should reach 99% recovery at a perfusion flow rate of 0.023 \( \mu L/min \), which is attainable by a normal syringe pump. Since the major mass transfer limitation is the diffusion through the polycarbonate membrane, the time for the system to come into equilibrium with the surrounding fluid \( t_{ss} \) can be estimated as

\[ t_{ss} \approx d^2 \frac{1}{D_m} \]  

Equation 3.42
The $t_s$ calculated for the ions is 24.4 s ($d$ is 6 µm for the polycarbonate membrane). This means that a rapid channel equilibrium is possible for rapid continuous sensing. Real time ion monitoring was possible due to the in-line sensing electrodes within the microfluidic channel. The predicted outputs from the step change and sinusoidal continuous monitoring experiments followed the functionality of the input signal with an appropriate phase lag and amplitude reduction. These results not only show good agreement with analytical predictions but also the stability of the device over long periods of time.

The phase lag seen in the step and sinusoidal monitoring was about 230 and 210 seconds. The major contribution to this lag was mostly due to the dead volume within the tubing between the syringe pumps and the microsystem, which was measured to be approximately 150 seconds, and not due to mass transfer limitations. As a result, future studies should include a built-in mixer within the PDMS layer to minimize the tubing dead volume and hence this lag time.
Chapter 4

On-Chip Microdialysis System with Glucose Sensing Capabilities

In this chapter, an electrochemical glucose sensor is integrated with the microdialysis system. Instead of gold (Au) impedance electrodes, platinum (Pt) and silver/silver chloride (Ag/AgCl) electrodes are used to determine the glucose concentration of the dialysate at the outlet of the microdialysis channel. The device was demonstrated for continuous glucose microdialysis and sensing. A Freestyle glucose monitor was used as a reference for determining the glucose concentration.

4.1 Glucose Sensing Theory

An enzyme-based sensor is described here to be integrated with the microdialysis system. An enzyme is a biological substance which recognizes specific molecules and transforms the molecules into quantifiable products. In enzymatic reactions, the substrate is transformed into the products according to the general reaction.

\[ E + S \xrightleftharpoons[k_{-1}]{k_1} ES \xrightarrow{k_2} E + \text{product(s)} \]  

Equation 4.1

The reaction rate of Equation 4.1 is induced from Michaelis-Menten kinetics and described as

\[ \text{Rate} = \frac{d[p]}{dt} = \frac{v_{\text{max}} [S]}{K_M + [S]} \]  

Equation 4.2

where \( v_{\text{max}} = k_2 [E]_0 ([E]_0 : \text{initial concentration}) \), \( K_M = (k_2 + k_{-1}) / k_1 \).
The catalysis of glucose by glucose oxidase (GOx) is as

\[
\text{Glucose} + O_2 \xrightarrow{\text{GOx}} \beta\text{-gluconolactone} + H_2O_2 \quad \text{Equation 4.3}
\]

\[
\beta\text{-gluconolactone} + H_2O \rightarrow \text{gluconic acid} \quad \text{Equation 4.4}
\]

In a kinetically controlled process, the enzymatic reaction is not fast enough to catalyze all the substrate molecules diffusing through the membrane. The kinetics of the enzyme reaction is the rate-determined step for the overall process at low enzyme concentrations. In practice, this is usually avoided by using sufficiently high enzyme concentration. Therefore, the reaction is very rapid and will not be the rate-limiting step for these applications.

From the results of this chemical reaction, a glucose sensor can be designed based on oxygen consumption\[83], local pH change\[84-86], or hydrogen peroxide detection as well[87-89]. The first glucose sensor developed by Updike and Hick was based on oxygen consumption[90]. A completely different electrochemical sensor is the ion-sensitive field effect transistor, or ISFET, which was converted into an enzyme field-effect transistor (ENFET) by Bergveld in 1970[91]. The generated H⁺ ion is correlated with the concentration of glucose and will result in a local pH decrease which in turn can be monitored by the underlying ISFET.

Hydrogen peroxide is also produced during the enzymatic reaction. The hydrogen peroxide concentration will increase linearly with increasing glucose concentration. A Pt electrode that is biased at +0.7 V versus a reference electrode (Ag/AgCl) can be used as hydrogen peroxide detector by oxidizing hydrogen peroxide at the working electrode surface while the AgCl is consumed at the cathode.
In this work, a hydrogen peroxide sensor is chosen to be integrated with the microdialysis chip because it can easily be integrated using the same thin-film based fabrication. After enzymatic catalysis, the hydrogen peroxide concentration is proportional to the glucose concentration. Amperometry, utilizing a Pt working electrode biased at +0.7V versus a reference electrode (Ag/AgCl) is the hydrogen peroxide detection method.

Amperometry is a voltammetric technique where the potential is kept constant. The measured current is a linear function of the flux of the electroactive species involved to the electrode surface. For infinite electrode area, the current is described by the Cottrell equation[92]:

$$i(t) = \frac{nFAD^{1/2}C}{\pi^{1/2}t^{1/2}}$$  \hspace{1cm} \text{Equation 4.7}

where $A$ is the electrode area, $D$ is the diffusion coefficient and $C$ is the bulk concentration of the oxidized molecules.

For real electrodes, a limiting current is reached and the current is proportional to the species concentration. For a spherical electrode, the current at the electrode is defined by spherical diffusion and found to be

$$i(t) = \frac{nFAD^{1/2}C}{\pi^{1/2}t^{1/2}} + 4\pi nFDC$$  \hspace{1cm} \text{Equation 4.8}

A three-electrode system is designed for recording the oxidation current. The configuration is shown in Figure 4-1[92]. The electrodes, from right to left, are working
electrode (WE), reference electrode (RE) and counter electrode (CE). The reference electrode is connected to a high impedance opamp, which results in no current flowing through the reference electrode. The counter electrode injects current into the solution to balance the electrochemical reaction. Thus, the applied potential at working electrode is well defined and not influenced by the current density. The current measured is then linearly proportional to the concentration of hydrogen peroxide.

Figure 4-1: The three-electrode voltammetric configuration
4.2 Device Fabrication

Similar to the impedance device introduced in Chapter 3, this microdialysis system with glucose sensing capabilities is composed of the microdialysis monitoring chip and a PDMS reservoir channel/mixer piece. Instead of gold impedance electrodes, Pt electrodes are sputtered within the fluidic channel of the microdialysis monitoring chip. Figure 4-2 displays a finished device with a Ag/AgCl pellet inserted into the sensing area as the reference electrode for glucose sensing.

![Figure 4-2: Schematic of the stacked microdialysis system with glucose sensing electrodes.](image)

4.2.1 Microdialysis Monitoring Chip

The Pt sensing electrodes were first fabricated using lift-off techniques on clean glass slides. The mask of the electrodes is shown in Figure 4-3. The areas of the working and the
counter electrode are 2.94 mm² and 2.1 mm². The contact pads are 2 mm x 2 mm, which was
designed to be large enough for easy wire connection. This is a dark field mask. The positive
photoresist of the white electrode areas exposed to UV light will be washed away during the
development.

The comprehensive procedures for positive photoresist defining the electrode areas
are described in Table 3-1 of section 3.2.1. After the photolithography procedures, the glass
slides were cleaned by dipping in 10% hydrofluoric acid solution for 5 seconds and rinsing
under running deionized (DI) water. A 100 Å thick Titanium/1000 Å Platinum layer was
then sputtered on the HF-cleaned glass slides. The glass slides were placed in an acetone
solution until all the unwanted Ti/Pt peeled off. Access holes for the fluidic connections
within the device were drilled by estimation before constructing the fluidic channels.

Figure 4-3: Mask for glucose sensing electrodes.
SU-8 2010 negative photoresist is the material chosen for the fluidic channels. The glass slides were again cleaned before applying SU-8. The mask of the fluidic channel is shown in Figure 4-4, in which the width of the channel design (a) is 700 µm and of design (b) is 800 µm with perfusion flow inlet and outlet of 1.5 mm x 1.5 mm square. This is a bright field mask. SU-8 of the dark channel area, not exposed to UV light, is washed away during the development. The microfluidic channel was determined to be 15 µm thick after the standard SU-8 fabrication procedures, shown in Table 3-2.

![Figure 4-4: Mask for SU-8 microfluidic channel of glucose sensing](image)

Polycarbonate track-etch membrane (pore size 15 nm and 100 nm, 6µm thick, Whatman Inc) was bonded with the SU-8 fluidic channel with the same treatment and procedures described in section 3.2.1.
4.2.2 PDMS Piece

In order to create an environment for testing the device in the time domain more efficiently, a stacked system was designed to fit on the microdialysis chip. The designs of the PDMS reservoir channel are shown in Figure 4-5. The diffusional area is 7 mm$^2$ in design (a) and 15.6 mm$^2$ in design (b). Aligning the two layers to bond together can be done by the naked eyes as well. The bonding procedures are exactly the same as described in chapter 3.

---

Figure 4-5: Designs for PDMS reservoir/mixer channel layer.
Standard soft lithography procedures were employed by using SU-8 2010 for the mold of 20 μm deep reservoir fluidic channels (Table 3-3). PDMS was poured onto the mold to create the desired piece. After curing, access holes for inlets, outlet and Ag/AgCl insertion were drilled on the PDMS piece.

The last step to finish this device is to assemble the Ag/AgCl electrode with the chip. Ag/AgCl pellet electrodes of 0.8 mm diameter were obtained from A-M systems. The pellet was inserted into the glucose sensing area directly as shown in Figure 4-2. Two pictures of an actual device are shown in Figure 4-6. The Pt electrodes are in the bottom covered with the SU-8 microfluidic channel layer, the polycarbonate membrane and the PDMS reservoir channel layer.

Figure 4-6: Pictures of an actual glucose microdialysis chip
4.3 Experimental Setup

The complete experimental setup is shown in Figure 4-7. A CHI electrochemical station, model type 750B, is used to collect the oxidation current for determining the glucose concentration in the sensing area. The red wire is the working electrode, the green is the counter electrode, and the black wire is the reference electrode. The same previously designed LabView program controlled the concentration in the PDMS reservoir channel by varying the relative flow rates of the two syringe pumps. The third syringe pump was used to produce perfusion flow. A PBS solution was mixed with glucose oxidase as the perfusion fluid. The perfusate contained glucose oxidase at a concentration of 1000 unit/ml mixed within 5 ml of PBS solution.

Despite the fabrication of a built-in mixer with the PDMS reservoir channel piece, an external mixing component was still used due to the high channel pressure within the integrated mixer causing the PDMS reservoir piece to delaminate from the membrane while infusing the glucose and PBS solutions directly into the top PDMS reservoir layer of the microdialysis chip. The reservoir solution was still mixed outside of the chip and then connected to the channel at the starting point of the diffusional area.
Packaged software provided by CH Instruments was used to record the oxidation current. The technique of amperometric i-t curve was chosen with electrode preconditioning steps, at a WE potential of 1.5 V for 30 seconds, -0.8 V for 20 seconds and 0.2 V for 5 second, followed by the experimental recording at the potential of +0.7 V. A representative data set from the software for a glucose concentration of 265 mg/dL is shown in Figure 4-8.

Figure 4-7: Experimental setup for glucose sensing.
Figure 4-8: A representative graph for a glucose concentration of 265 mg/dL recorded from the CH Instruments packaged software.
4.4 Results and Discussion

A Therasense Freestyle glucose monitor was employed to determine glucose concentration as a reference. The glucose microdialysis was first conducted using the track-etch polycarbonate membrane with 15 nm diameter pores. The glucose recovery was too low to be determined by the glucose monitor due to the low glucose permeability of the membrane. Therefore, the device was subsequently integrated with the track-etch polycarbonate membrane of 100 nm diameter pores. The device was demonstrated with continuous glucose microdialysis and sensing.

4.4.1 Glucose Microdialysis with Polycarbonate Membrane with 15 nm Diameter Pores

Glucose recovery was initially tested with a 15 nm diameter pore size polycarbonate membrane, but the recovery was lower than 4% even at a perfusate flow rate of 0.2 µL/min with a reservoir concentration of 500 mg/dL. The glucose monitor was not able to determine the concentration since it was lower than 20 mg/dL, the lower limit for the monitor. Since the permeability of glucose to the membrane is so low, it would be impossible to obtain high recovery with the design of the microdialysis system. As a result, the same polycarbonate membrane with larger pores, 100 nm diameter pores, was chosen to be integrated with the microdialysis system.
4.4.2 Glucose Microdialysis with Polycarbonate Membrane with 100 nm Diameter Pores

Thin-film platinum electrodes were used here for glucose sensing based on the detection of hydrogen peroxide. Pt has the advantage that it is less reactive towards the Cl\(^-\) ions in the electrolyte under a positive potential bias while Au and Ag electrodes will chloridate under these conditions\cite{92}. The adhesion of Ti/Pt thin films on the glass slides was consistently good. The relationship between the anodic oxidation current and the glucose concentration was obtained over the experimental operating range. The measured solution conductance as a function of PBS concentration using the microdialysis system with impedance sensing electrodes is shown Figure 4-9.

\begin{figure}[h]
\centering
\includegraphics[width=0.7\textwidth]{glucose_solution_calibration.png}
\caption{Glucose concentration v.s. Oxidation current}
\end{figure}
The calibration is divided into two segments. The slope is larger in the more diluted region, and it then decreases as the glucose solution becomes more concentrated. The overall sensing process is influenced by the kinetics of the reaction, by diffusion of the involved elements toward the electrode and by stoichiometric limitations. In a kinetically controlled process, the enzymatic reaction is not rapid enough to oxidize all the glucose in the solution. Kinetic control usually takes place at low enzyme concentrations. The volume between the microdialysis area and the sensing area was about 0.15 \( \mu \text{L} \). It took less than 20 seconds for the perfusion flow to reach sensing area at the experimental flow rate of 0.5 \( \mu \text{L/min} \). Despite the high enzyme concentration used in this work, it was still possible that the enzymatic reaction was not sufficiently fast enough to convert all the glucose into hydrogen peroxide. Diffusional control also occurs when the electrodes are covered with a low permeability membrane. Since the electrodes were not covered with membrane, diffusional control should not play a role here. The reaction can also be limited by stoichiometric restrictions. If all the oxygen in the flow is consumed, the oxidation current does not reflect the true glucose concentration and are governed by the oxygen concentration. Oxygen deficiency results in saturation of the sensor at high glucose concentrations.

The system was tested for glucose dialysis. The PDMS reservoir channel was infused with glucose solution at a concentration of 398 mg/dL at constant flow rate of 10 \( \mu \text{L/min} \). The diffusional area was 7.28 mm\(^2\). The permeability was calculated to be 5.44 \( \mu \text{m/s} \) with a standard deviation of 0.71 \( \mu \text{m/s} \) (Table 4-1). Glucose recovery as a function of perfusion flow rates was obtained in Figure 4-10. Recoveries at high perfusion flow rates were not performed here due to the following two reasons: First, the pressure at high perfusion flow
rates might be high enough to cause the membrane to delaminate; Second, the perfusion flow rates have to be negligible compared to the flow rate of the PDMS reservoir channel in order to satisfy the assumption for Equation 3.19. The highest recovery was 99% at a perfusate flow rate of 0.5 µL/min.

Table 4-1: Glucose microdialysis results

<table>
<thead>
<tr>
<th>Experiment</th>
<th>$K_0A_m$(µL/min)</th>
<th>$K_0A_n$(µm3/s)</th>
<th>$K_0$(µm/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Set 1</td>
<td>2.302</td>
<td>38363333.33</td>
<td>5.27</td>
</tr>
<tr>
<td>Set 2</td>
<td>2.362</td>
<td>39363333.33</td>
<td>5.41</td>
</tr>
<tr>
<td>Set 3</td>
<td>2.796</td>
<td>46598333.33</td>
<td>6.40</td>
</tr>
<tr>
<td>Set 4</td>
<td>2.046</td>
<td>34100000</td>
<td>4.68</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
<td>5.44</td>
</tr>
<tr>
<td>STD</td>
<td></td>
<td></td>
<td>0.71</td>
</tr>
</tbody>
</table>
Figure 4-10: Glucose microdialysis recovery
Subsequently, experiments monitoring glucose concentration in the time-domain were conducted. The device reservoir concentration was controlled temporally by controlling the two syringe pumps each with a different glucose concentration through a LabView program. By varying the relative infusion rate between each pump and mixing the incoming solutions different glucose reservoir concentrations were possible.

First, the step response of the microdialysis system was collected. The flow rate of syringe pump 1 (398 mg/dL) was initially 3 µL/min, raised up to 7 µL/min at the tenth minute and then reduced to 3 µL/min at the twentieth minute. The flow rate of syringe pump 2 (PBS solution) was 180 degrees out of phase, shown in Figure 4-11. The total flow rate of PDMS reservoir channel was kept at 10 µL/min.

Therefore the reservoir concentration started at a glucose concentration of 119 mg/dL, went up to 279 mg/dL at the tenth minute and then came back down to 119 mg/dL again at the twentieth minute (Figure 4-12). The perfusion flow rate was maintained at 0.5 µL/min, which allowed a 99% recovery according to the recovery results in Figure 4-10.
The system response to any input waveform can be modeled using linear system theory by convolving the input waveform with the impulse response. For a given step response, \( g(t) \), the impulse response, \( h(t) \), could be obtained by taking the first derivative of the step response.

\[
h(t) = \dot{g}(t)
\]  

Equation 4.9

With the impulse response, the output signal can be predicted.

\[
\text{Signal}_{\text{out}} = \text{Signal}_{\text{in}} * h(t)
\]  

Equation 4.10

Next, the concentration of the reservoir channel was varied in a sinusoidal fashion from 80 mg/dL to 318 mg/dL by varying the relative flow rate of the two input syringe...
pumps while the total PDMS reservoir flow rate remained at 10 µL/min (Figure 4-13). The period of the input signal was 800 seconds with a concentration change every 40 seconds. This period was chosen because it is a sufficient time scale for tracking physiological concentration changes such as glucose fluctuations within a diabetic patient.

![Figure 4-13: Flow rate control for the sinusoidal input](image)

Figure 4-14 shows the sinusoidal response of the system. The experimental results and the predicted output were in good agreement with a negligible amplitude reduction. At the perfusion flow rate of 0.5 µL/min, the system was able to track the concentration waveform with a 99% recovery and a phase lag of 210 seconds. Only 45 seconds out of the 210 seconds phase lag was contributed by the microdialysis system. The rest was due to the tubing connection between the mixer and the microdialysis chip. Both input and output signal were converted into the frequency domain by Fourier transforms and had an obvious frequency peak at 0.00125 Hz, which represents the 800 second sinusoidal period. Matlab was used to process the data. A Pearson’s correlation coefficient (r) has been determined to be 0.99 between the experimental data and the system response predicted by linear systems.
The diffusion coefficient of glucose in bulk water is $9.09 \times 10^{-10} \text{ m}^2/\text{s}$. From Equation 3.30, the mixing time was calculated to be 1.76 second. Based on the volume of the mixer and the PDMS reservoir flow rate, it would take 2.52 seconds for fluid to flow through the fluidic channel of the mixer, so the solution exiting the mixer is considered well mixed.

The pressure drop along the microchannel can be neglected when compared with the atmospheric pressure. The osmotic pressure-induced flux was also found to be negligible when compared with the diffusional flux due to the concentration gradient.

Figure 4-14: The response of sinusoidal input demonstrated with the glucose microdialysis system
The permeability was obtained as 5.44 µm/s (STD = 0.71 µm/s, $D_m = 32.64 \mu m^2/s$) and it is in good agreement with the theory. The system reaches 99% recovery at a perfusion flow rate of 0.5 µL/min. The time for the system to come into equilibrium with the surrounding fluid $t_s$ can be estimated as

$$t_{ss} \approx \frac{d^2}{D_m} \quad \text{Equation 4.11}$$

The $t_s$, calculated for the ions is 1.1 s ($d$ is 6 µm for the polycarbonate membrane). This means that a rapid channel equilibrium is possible for rapid continuous sensing. Moreover, due to the in-line sensing electrodes within the microfluidic channel, real time glucose monitoring was demonstrated. The predicted outputs from the step change and sinusoidal continuous monitoring experiments followed the functionality of the input signal with an appropriate phase lag and a negligible amplitude reduction. These results not only show good agreement with analytical predictions but also the stability of the device.

The integration of the microdialysis chip with in-line sensing capabilities is meant to reduce the glucose sensing delay caused by the dead volume with a tubing assembly. The fluidic channel volume is about 0.15 µL from the microdialysis area to the glucose sensing area. It takes only 20 seconds for the perfusion flow to reach the sensing area at the perfusion flow rate of 0.5 µL/min. The recovery of 99% was obtained with the microdialysis system with a diffusional area of about 7 mm². The electrochemical sensing component was able to continuously track concentration changes in the reservoir. This system is expected to have the proper sensitivity to track physiologically relevant concentration changes of glucose (maximum change rate ~4 mg/dl-min with periodicity of 1 hour or greater) with minimal lag time and amplitude reduction for continuous glucose monitoring for diabetes treatment.
Chapter 5
Conclusions and Future Work

The complete fabrication protocol for the on-chip microdialysis system was developed. The track-etch polycarbonate membrane was successfully bonded with the SU-8 fluidic channels with high reproducibility, stability and no leakage. The disadvantage of the membrane is that the porosity seems to vary from batch to batch. When a new box of the membrane was integrated with the device, the permeability of the membrane to the same molecules under the same conditions was not consistent with the results from the previously used membranes. The technical information provided on the website of the manufacturer indicates the rated pore density is between $1 \times 10^5$–$6 \times 10^8$ pores/cm$^2$, which is an enormously wide range, and no further information about the membrane with a specific pore size could be obtained. As a result, the permeability of the membrane can not be predicted by

$$K_m \approx \frac{DF(a/r)p}{dT}$$  \hspace{1cm} \text{Equation 5.1}$$

where $D$ is the diffusivity of a species in water, $F(a/r)$ is a drag factor, $a$ is the molecular diameter, $r$ is the radius of the pores, $p$ is the porosity or volume fraction of pores versus total membrane volume, $d$ is the thickness of the membrane and $T$ is the tortuosity of the membrane[93] because both $p$ and $T$ are unknown. $F(a/r)$ is the centerline approximation for a spherical molecule and is estimated as

$$F(a/r) \approx 1 - 2.1044(a/r) + 2.089(a/r)^3 - 0.948(a/r)^5$$  \hspace{1cm} \text{Equation 5.2}$$

when $a/r < 0.4$[94]. The exact membrane properties must be determined by experiment.
Even though the device was designed for direct contact between dialysis membrane and tissue of interest, in order to create a reservoir environment with the concentration continuously being changed for testing the time resolution of the device, a stacked system and a micro-mixer were designed to cooperate with the microdialysis chip. A LabView program successfully and precisely controlled the solution concentration within the PDMS reservoir channel by varying the relative flow rates of the two syringe pumps. For impedance microdialysis part, the impedance data was recorded with the same flow rate controlling LabView program simultaneously. An experimental set-up was constructed to test the microdialysis system sufficiently.

As the first step toward the integration of a microdialysis system and a biosensor, the microdialysis chip was initially integrated with Au impedance sensing electrodes, then with a glucose sensor based on the same thin-film fabrication. The device was demonstrated and characterized by obtaining membrane permeability and recoveries at different perfusate flow rate. The integration of the microdialysis chip with in-line sensing capabilities was meant to reduce the glucose sensing delay caused by tubing assembly. The fluidic channel volume was about 0.15 µL from the microdialysis area to the glucose sensing area. It took only 20 seconds for the perfusion flow to reach the sensing area at the perfusion flow rate of 0.5 µL/min. The recovery of 99% was obtained with the microdialysis system of a diffusional area of about 7 mm². Compared with the commercially available microdialysis probes, the on-chip microdialysis system was able to obtain high recoveries with a smaller diffusional membrane area. The experimental data were in good agreement with analytical modeling. In time domain microdialysis experiments, the major contribution to the lag was mostly due to the dead volume within the tubing between the mixer and the microsystem, and not due to
mass transfer limitations. The electrochemical sensing component was able to continuously track concentration changes in the reservoir. The system is expected to have the proper sensitivity to track physiologically relevant concentration changes of glucose with minimal lag time and amplitude reduction for continuous glucose monitoring for diabetes treatment.

Several types of semipermeable membrane of different pore sizes or with different molecular weight cut off (MWCO) can be integrated, such as cellulose acetate, polyamide and polyethylene membrane. Bonding strength between the membranes and the SU-8 layer will be compared and the permeability of different membranes can subsequently be characterized. The purpose is to find appropriate membrane for sampling specific molecules in various environments with the microdialysis system.

A more sophisticatedly designed glucose sensing component is desired to be combined with the microdialysis system. The enzyme, glucose oxidase, can be immobilized on the electrodes instead of mixing with the perfusion flow. One of the most important things to immobilize an enzyme to a surface is to use an attachment method that will cause minimum loss of the enzyme activity and not change the chemical nature or reactive groups in the binding site of the enzyme. Conventional procedures of enzyme immobilization include cross-linking, covalent binding and entrapment in gels or membrane. Among the various methods, an entrapment in a polymer membrane is commonly used[95]. The membrane is formed from polymer solution in organic solvent. The method is a simple dipping of the electrode into the solution or casting a small volume of the solution onto the electrode surface[96]. Besides the conventional methods, immobilization using electropolymerized membrane results in polymer layers of controlled thicknesses and higher reproducibility[95, 97].
The reference Ag/AgCl electrode was external to the microdialysis chip, which added an uncertainty to the device due to lack of precision by manual insertion. The reference electrode can be also prepared as a thin film within the microfluidic channel. Silver layer is deposited assisted with the lift-off techniques. An intermediate layer of titanium or chrome is usually used to promote the adhesion on glass slides. Next, the electrode needs to go through a process, called chloridation, to convert Ag to Ag/AgCl. The chloridation can be done electrochemically in NaCl or HCl solution by applying a voltage of +0.7 V, or chemically by dipping in an FeCl₃ or KCrO₃Cl solution[92]. Another way to fabricate planar Ag/AgCl electrodes is the screen-printing method. A paste consists of a mixture of the material of interest, an organic binder, and a solvent is pressed into a substrate through the openings in the emulsion on a stainless steel screen. The pattern in the screen emulsion is transferred onto a substrate by forcing the paste through the mask openings with a squeegee. The resolution of the process depends on the openings in the screen and the nature of the pastes. The advantage of screen printing is the cost reduction to deposit a wide variety of films on planar substrates[98, 99].

In the future, the system should be used for biological studies in vivo such as implanting the probe in a diabetic animal model. Besides monitoring biomolecules on human and animals, the microdialysis technique is also utilized for environmental research. The probes are inserted into freshwater systems or soil for real-time and continuous sampling with minimum disturbance of the outer environment[34, 100].

Other types of amperometric biosensors can be integrated with the microdialysis chip since microelectrodes use the common photolithographic fabrication techniques which are already used to construct the microdialysis system. Additional integration with
micropumps, microvalves and other components are possible as well, leading to more self-contained µTAS systems.


VITA

Yi-Cheng Hsieh

Education:
2004~2006 Ph.D., Department of Bioengineering
Pennsylvania State University, State College, PA, USA
Dissertation Title: “On-Chip Microdialysis System with Flow-through Glucose Sensing Capabilities”.
Advisor: Jeffrey D. Zahn

2002~2004 M.S., Department of Bioengineering
Pennsylvania State University, State College, PA, USA
Thesis Title: “Glucose Recovery in a Microfluidic Microdialysis Chip”
Advisor: Jeffrey D. Zahn

1996~2000 B.S., Department of Civil Engineering
National Taiwan University, Taipei, Taiwan

Awards:
• Gold prize in the Peterson Student Research Prizes at the Diabetes Technology Meeting 2006 for the abstract “On-Chip Microdialysis System with Flow-Through Glucose Sensing Capabilities”.
• Travel award at the 18th IEEE international conference on MEMS, 2005, Miami Beach, FL, USA for “On-Chip Microdialysis System with In-Line Sensing Capabilities”.

Journal Publication:

Book Chapter:
• “Microdialysis” submitted to Encyclopedia of Micro and Nanofluidics, Springer eBook Collection in Engineering.