Overcoming Limitations of Iontronic Delivery Devices

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Description of the cover image:

The illustration of the cover is designed by Robert Brooke and depicts a fiber capillary delivery device for modulation of inflammation in human monocytes.

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During the course of the research underlying this thesis, Maria Seitanidou was enrolled in Forum Scientium, a multidisciplinary graduate school at Linköping University, Sweden.

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To my family

Abstract

Organic electronic devices are considered as one of the best candidates to replace conventional inorganic electronic devices due to their electronic conductive functionality, low-cost production techniques, the ability to tune their optical and electronic properties using organic chemistry, and their mechanical flexibility. Moreover, these systems are ideal for bioelectronic applications due to their softness, biocompatibility, and most importantly, their electronic and ionic transport. Indeed, these materials are compatible with biological tissues and cells improving the signal transduction between electronic devices and electrically excitable cells. As ions serve as one of the primary signal carrier of cells, they can selectively tune a cell's activity; therefore, an improved interface between electronics and biological systems can offer several advantages in healthcare, e.g. the development of efficient drug delivery devices.

The main focus of this thesis is the development of electronic delivery devices. Electrophoretic delivery devices called organic electronic ion pumps (OEIPs) are used to electronically control the delivery of small ions, neurotransmitters, and drugs with high spatiotemporal resolution. This work elucidates the ion transport processes and phenomena that happen in the ion exchange membranes during ion delivery and clarifies which parameters are crucial for the ion transport efficiency of the OEIPs. This thesis shows a systematic investigation of these parameters and indicates new methods and OEIP designs to overcome these challenges. Two novel OEIP designs are developed and introduced in this thesis to improve the local ion transport while limiting side effects. OEIPs based on palladium proton trap contacts can improve the membrane permselectivity and optimize the delivery of γ -aminobutyric acid (GABA) neurotransmitters at low pH while preventing any undesired pH changes from proton transport in the biological systems. And OEIPs based on glass capillary fibers are developed to overcome the limitations of devices on

planar substrates, related to more complex and larger biologically relevant ion delivery with low mobility for implantable applications. This design can optimize the transport of ions and drugs such as salicylic acid (SA) at low concentrations and at relatively much higher rates, thereby addressing a wider range of biomedically relevant applications and needs.

Populärvetenskaplig Sammanfattning

Läkemedel utgör det klassiska terapeutiska tillvägagångsättet till att behandla sjukdomar. Nya framtagna aktiva substanser kan verka väldigt lovande i förkliniska undersökningar, men senare misslyckas i de kliniska testerna på grund av deras giftighet, sidoeffekter, för snabb nedbrytning eller misslycka att nå det önskade behandlingsområdet i kroppen. Vanligtvis så brukar inte läkemedel administreras lokalt till det specifika området i kroppen som är påverkad av sjukdom, vilket kan ge skadliga konsekvenser i andra områden. I fallet av neurologiska sjukdomar är situation ännu mer komplex, där läkemedlet behöver passera blod hjärnbarriären innan det kan nå det specifika målet i hjärnan. Ytterligare så kan transportmekanismer aktivt transportera läkemedlen tillbaka ut i blodomloppet vilket kan begränsa dess effekt i det centrala nervsystemet. Nya strategier har utvecklats för att undkomma dessa begränsningar i behandlingsområdet för neurologiska sjukdomar, som kan tillåta ingripande för behandling på begäran precist vart och när det behövs. I den närmsta framtiden så är läkemedel fortfarande det bästa behandlingssättet; där av måste framstegs göras för att undkomma de ovannämnda problemen och utmaningar.

Organisk elektronik anses vara en av de bästa kandidaterna till att förbättra livskvalitén för patienter som undergår olika former av behandlingar. Detta kan ske tack vare deras elektroniska ledningsförmågas egenskaper, låga tillverkningskostnad, förmågan att justera dess optiska och elektroniska egenskaper med hjälp av organisk kemi och dess mekaniska flexibilitet. Dessutom så är de här systemen ideala för bioelektroniska applikationer tack vare deras mjukhet, biologiska kompatibilitet och viktigaste av allt, deras elektron- och jontransportförmåga. Dessa material som är kompatibla med biologiska vävnader och celler, kan förbättra signal överföringen mellan elektronik och elektriskt stimulera bara celler. Då joner fungerar som en av de huvudsakliga signalbärarna till celler, kan de selektivt stimulera en cells aktivitet; där av ett förbättrat gränssnitt mellan elektronik och biologiska

system som kan erbjuda flera fördelar inom sjukvård, t.ex. utvecklingen av effektiv och selektiv läkemedelsdoseringsteknologi.

En ideal lösning skulle vara att kunna leverera läkemedel precis där de behövs på begäran. I den här kommunikationen använder vi oss av organiska elektroniska jonpumpar (OEJP) för att leverera aktiva substanser till vävnader. OEJP används till att elektroniskt kontrollerbart leverera små joner, signalsubstanser och läkemedel med hög precision och doseringsnoggrannhet. Det här arbetet klargör jontransportprocesser vid användandet av OEJP och fenomen som sker i jonutbytesmembran vid transport av joner, läkemedel och signalsubstanser som är väsentliga för effektiv jontransport hos OEJP. Avhandlingen visar en systematisk undersökning av dessa parametrar och indikerar till nya metoder och designer för att överkomma dessa utmaningar. Två originella OEJP designer har utvecklats och introduceras i den här avhandlingen till att förbättra den lokala jontransporten samtidigt som sideoeffekter har minimerats. OEJP baserade på palladium protonfällor kontakter kan förbättra membranselektivitet och optimera doseringen av signalsubstansen gammaaminosmörtsyra (GABA) vid lågt pH, samtidigt som oönskade pH förändringar från protontransport förhindras i det biologiska systemet. Jonpumpar baserade på glasskapillärer har utvecklats för att överkomma begränsningar med enheter på planasubstrat, som är relaterade till mer komplexa och större joner som har låg mobilitet och är biologiskt relevanta till implanterbara applikationer. Den här designen kan optimera transporten av joner och läkemedel så som salicylsyra (SA) vid låga koncentrationer och med relativt snabbare dosering, där igenom hantera ett bredare spektrum av biomedicinskt relevanta applikationer och behov.

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List of Included Publications

Paper I:

pH–Dependence of Gamma-Aminobutyric Acid Iontronic Transport

Maria Seitanidou, Juan Felipe Franco-Gonzalez, Theresia Arbring Sjöström, Igor Zozoulenko, Magnus Berggren, Daniel T. Simon

Journal of Physical Chemistry B. **2017**, 121, 7284.

Contribution to this Paper: Developing of fabrication techniques, performing electrical and chemical characterization, analysis and evaluation of the results, and writing the manuscript.

Paper II:

A proton trapping ion pump for selective drug delivery

Maria Seitanidou, Xenofon Strakosas, Daniel T. Simon, Magnus Berggren

(manuscript in preparation)

Contribution to this Paper: Developing of fabrication techniques, performing electrical and chemical characterization, analysis and evaluation of the results, and writing the manuscript.

Paper III:

Overcoming transport limitations in miniaturized electrophoretic delivery devices

Maria Seitanidou, Klas Tybrandt, Magnus Berggren, Daniel T. Simon

Lab on Chip. **2019**, 19(8).

Contribution to this Paper: Developing of fabrication techniques, performing electrical and chemical characterization, analysis and evaluation of the results, and writing the manuscript.

Paper IV:**Modulating inflammation in monocytes using organic electronic fiber capillary ion pumps**

Maria Seitanidou, Robert Blomgran, Giggil Pushpamithran, Daniel T. Simon Magnus Berggren

Advanced Healthcare Materials. 2019, 1900813

Contribution to this Paper: Developing of fabrication techniques, performing electrical and chemical characterization, analysis and evaluation of the results, and writing the manuscript

Related Work Not Included in the Thesis

Review:

A Decade of Iontronic Delivery Devices

Theresia Arbring Sjöström^a, Magnus Berggren^a, Erik O. Gabrielsson^a, Per Janson^a, David J. Poxson^a, Maria Seitanidou^a, Daniel T. Simon^a

Advanced Materials Technologies. 2018,3,5

Paper I:

Treatment of Brain Tumor Cells with Organic Electronic Ionic Pumps

Linda Waldherr^a, Maria Seitanidou^a, Marie Jakešova, Marilena Knittelfelder, Clemens Stilianu, Tony Schmidt, Romana Schober, Nassim Ghaffari Tabrizi-Wizsy, Silke Patz, Daniel Simon, Rainer Schind

(manuscript in preparation)

Paper II:

Organic Electronic Ion Pumps for Chemical Actuators

Johannes Bintinger^a, Maria Seitanidou^a, David Poxson, Hannes Mikula, Magnus Berggren, Daniel Simon

(manuscript in preparation)

Paper III:

Conductivity-Structure Relation of Hyperbranched Polyelectrolytes

Tobias Abrahamsson, Mikhail Vagin, Arghyamalya Roy, Maria Seitanidou, Ioannis Petsagkourakis, Jaywant Phospase, Nathalie Moro, Daniel T. Simon, Magnus Berggren

(manuscript in preparation)

Acronyms

OEIP	Organic electronic Ion Pump
CP	Conducting Polymer
IEM	Ion Exchange Membrane
CEM	Cation Exchange Membrane
AEM	Anion Exchange Membrane
$C_{counter}$	Counter Ions Concentration
C_{co}	Coions Concentration
C_{fixed}	Concentration of fixed charges
D	Diffusion coefficient
μ	mobility
S	Permselectivity
E	Electric Field
J	Diffusion Flux
t_i	Ion Transport Number
φ_{Don}	Donnan Potential
φ_{Dif}	Junction Potential

H^+	Proton
OH^-	Hydroxide
NaCl	Sodium Chloride
HCl	Hydrochloric Acid
KCl	Potassium Chloride
KOH	Potassium Hydroxide
GABA	γ -aminobutyric acid
Glu	Glutamate
AChCl	Acetylcholine
SA	Salicylic Acid
ASA	Acetylsalicylic Acid
SCR	Space Charge Region
I_{lim}	Limiting Current
PSS-co-MA	poly (4-styrenesulfonic acid-co-maleic acid)
AMPSA	2-acrylamido-2-methylpropane sulfonic acid
AETMAC	2-(acryloyloxy)ethyl trimethylammonium chloride
PEG	polyethylene glycol
PEG-DA	polyethylene glycol diacrylate
GOPS	3-glycidoxypropyltrimethoxysilane

PMMA	poly (methyl methacrylate)
Ag/AgCl	Silver/Silver Chloride
Au	Gold
Cr	Chromium
Ti	Titanium
Pd	Palladium
PdNPs	Palladium Nanoparticles
UV	Ultraviolet
RIE	Reactive Ion Etching
RF	Radio Frequency
ELISA	enzyme-linked immunosorbent assay
MD	Molecular Dynamics
DFT	Density Functional Theory
ESP	Electrostatic Potential
DMEM	Dulbecco's Modified Eagle Medium
LPS	Lipopolysaccharides
TNF- α	Tumor Necrosis Factor-alpha
IL-6	Interleukin-6

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Part I

Background

1

Introduction and Aim of Thesis

1.1 Organic Electronics

The field of Organic Electronics is based on the fabrication of electronic devices that are composed of organic macromolecules that are characterized by the backbone chain of alternating double- and single-bonds (conjugated polymers) with desirable electrical properties, such as high electronic and ionic conductivity. Organic electronic materials, in comparison to conventional inorganic (semi-)conductors, are built from organic (carbon-based) small molecules or polymers using synthetic strategies developed in the context of organic and polymer chemistry. In principle, a polymer is a macromolecule composed of repeat subunits and is generally electrically insulating.

However, in the 1970s, it was proven that polymers can actually exhibit high electrical conductivities, via chemical doping.^{1,2} Additionally, organic electronic devices are advantageous with respect to their inorganic counterparts due to their possibility of tuning their optical and electrical properties through polymer synthesis³, their low-cost fabrication process and their mechanical stability and flexibility.⁴⁻⁶ As a result, the academic and industrial interest has shifted towards organic electronics for applications,⁷⁻⁹ such as organic light-emitting diodes (OLEDs),^{10,11} organic photovoltaic solar cells (OPVs),^{12,13} organic electrochemical transistors (OECTs),¹⁴ and organic field-effect transistor (EGOFETs).^{15,16} In these devices, ions play a major role and the device functionality is defined by the ionic / electronic coupling of ionic and electrical signals in conductive polymers. These unique properties that combine ionic and electronic conductivity and electrochemical properties of conjugated polymers make organic electronics suitable for interactions with biological systems and enable the development of new technology for bioelectronic applications: organic bioelectronics.

1.2 Organic Bioelectronics

In 1780 at the University of Bologna, Luigi Galvani performed the first study of bioelectricity in animals. The bioelectricity field examines the electrical signals from tissues such as nerves and muscles.¹⁷ Galvani discovered that applying electrical sparks to muscles of the frog's legs can provoke a twitching of the legs. Further experiments confirmed this effect and Galvani was convinced that he was investigating the effect of animal electricity, the life force within the muscles of the frog. In 1792, Alessandro Volta at the University of Pavia found that the electricity was produced by the two dissimilar metals and not by the frog's legs. The twitching of the frog's legs was an indicator of the presence of the electricity. These studies were the starting point of electronic stimulation of the biological system and recording of neuronal signals and it can be considered as the first organic bioelectronic experiment.

Organic bioelectronics is a unique signal translator between biology and electronics and acts as a technological solution to a variety of diagnostic and therapeutic purposes.^{18–21} During the last decade, organic bioelectronic systems have been used to record and regulate biological functions overcoming pharmaceutical or traditional bioelectronics techniques.^{22,23} This technology can serve as an efficient tool for applications such as biosensing,^{24–26} electrophysiological recording,²⁷ and drug delivery.^{28–30} The organic electronic ion pump (OEIP) is a fundamental component of organic bioelectronics, which combines electronic and ionic properties of organic electronics,^{31,32} to effect release, via electronic addressing, of ionic-biochemical signals for biological applications.

1.3 Advantages and Properties of Organic Bioelectronics

Organic bioelectronics show great potential for applications in biomedical science and medicine since conjugated polymers bridge the gap between electronics and biology by being electronically and ionically conductive. Incorporation of biomolecules into the films can promote adhesion or biocompatibility. The biocompatibility,³³ and the softness,³⁴ of conducting polymers also make organic bioelectronics a good candidate material for various biomedical applications. Additionally, the advantages of their mechanical stability and flexibility make them suitable for contact with tissues and cells. The conducting polymers can be made even more attractive for their intended biological environment via chemical tailoring by decoration with suitable biomolecules.³⁵

1.4 State of the Art Bioelectronic Techniques

Despite a variety of alternative technologies, organic bioelectronics offer unique methods for the delivery of therapeutic substances and an ideal foundation for the treatment of various disorders, bridging the signalling gap between biology and technology.^{19,20,36,37} State of the art bioelectronic technologies allow in vivo and in vitro recording of neural activity, monitoring and manipulating of neural pathways, use of chemicals naturally occurring in the non-disease state, immediate treatment by self-regulation, precise control of substance delivery, and minimization of adverse effects arising from disturbance of the biological microenvironment by diffusive delivery.^{22,38} State of the art bioelectronic devices have been developed providing a fundamental understanding of communication between cells and with their environment.^{39–41} During the last decade, biosensors such as glucose monitors for diabetics,^{42,43} pacemakers,^{44,45} and cochlear implants,^{46,47} for damaged physiological functions are considered as successful therapies since the patient can return to a healthy lifestyle.

1.5 Aim of Thesis

The aim of this thesis is to develop electronic delivery devices based on the attractive properties of electrically conducting polymers. In this thesis, i have focused on the fundamental understanding of device physics of planar organic electronic ion pumps (OEIPs) and capillary fibers OEIPs which enable drugs and neurotransmitters to be locally delivered with high spatial and temporal resolution. I have developed a systematic investigation of parameters that can affect and limit the ion transport efficiency and indicated methods to overcome these challenges.

1.6 Thesis Outline

The thesis consists of two parts. The first part (Part I) of this thesis contains 7 chapters. Chapter 1 and Chapter 2 describe the background to the field of organic electronics and organic bioelectronics, with a focus on iontronic components and applications. Chapter 3 focuses on the ion transport process and ion-exchange membranes, which are the fundamental components of organic electronic ion pumps (OEIPs). Chapter 4 emphasizes the background and function of OEIP devices used in the included papers. Chapter 5 describes the fabrication and characterization of the devices presented in Chapter 4. Chapter 6 presents an overview of the publications.

The second part (Part II) of this thesis, includes the scientific results of this work in the form of publications. Chapter 7 gives a brief conclusion and a future perspective. Paper I presents an investigation of the role of pH on OEIP transport efficiency using the neurotransmitter γ -aminobutyric acid (GABA) as the model cationic delivery substance. Paper II presents the development of OEIPs incorporating palladium proton traps that improve the delivery rate and selectivity of GABA neurotransmitter delivery at low pH. Paper III presents the development of a new OEIP design based on capillary fibers, and challenges and improvements for low dose delivery for implantable applications. Finally, Paper IV presents the use of capillary fiber OEIPs in modulating inflammation in primary human monocytes in vitro.

2

Organic Bioelectronic Actuators & Delivery Devices

2.1 Actuators

An actuator is a component of a device or system that is responsible for moving and controlling a mechanism or system by generating a force, motion, heat and/or flow. Actuators are for instance, used in bioelectronics systems to cause stimulation of biological systems and to trigger desired signals or processes.

2.1.1 Electronic Stimulation

Actuators have been extensively used to stimulate and trigger functions of biological systems such as in neurons and muscle cells by means of an electric field generated from electrodes. Applying a potential at the electrodes causes the cell membrane to depolarize, which then triggers ion flows across the membrane achieving the electrical stimulation of cells.⁴⁸ The impact of stimulation depends on the applied potential, the distance between the electrode(s) and the targeted cells and the size and capacitance of the electrodes. The potential range should be within the polarizable regime to avoid any toxic electrochemical side reactions.⁴⁹ The distance between the electrodes and the targeted cells should not exceed the electrode size, otherwise, the effect on the cells might be limited due to the divergence of electric field lines. In order to increase the spatial resolution of the individual electrode or for instance a whole matrix of many electrodes, the size of the electrodes should be reduced.⁵⁰ Conducting Polymer (CPs) electrodes having a large capacitance and low impedance, offer several advantages as the active

material in actuator applications. Their softness and flexibility as compared to metal electrode counterparts make them more suitable for an actuator-tissue system. They are often used as stimulating electrodes in neuronal applications.⁴⁹

2.1.2 Mechanical Actuators

CPs are typically used in polymers actuators that are commonly applied to biomedical and biotechnology applications. CPs can be used as volume-changing materials since they undergo morphological changes during oxidation and reduction. During electrochemical doping/de-doping (oxidation/reduction (redox)) of a CP in an electrolyte, ions are exchanged between the electrolyte and the CP to maintain charge balance,⁵¹ upon redox switching.

The polymer synthesis is a critical parameter for the actual mechanism since the volume change depends on the kind of dopant ions species of the polymer.^{52,53} The redox-driven CP volume change mechanism is represented by two different cases. In the case that the polymer is synthesized with mobile doping ions, the mobile ions can migrate in and out of the polymer during the doping or de-doping process. Applying a potential to the CP, electrochemical doping or de-doping occurs, which results in an increase or decrease of the number of counter-balanced charges in the CP, respectively. The actuator volume is increased during doping (oxidation) since electrons are extracted from the CP and counterions from the electrolyte drift into the CP bulk. The polymer then swells as a result of that counterions and solvent enter the bulk. During the de-doping (reduction) process the polymer bulk instead shrinks to expel solvent and counterions (Figure 2.1.a).⁵⁴

In the case that the polymer contains immobile (polymer) doping ions, the opposite phenomenon typically occurs as a potential applied. The volume of the actuator instead decreases during oxidation and increases during the reduction process since electrons are injected to the CP and the immobile ions cannot escape the polymer. Upon bringing the CP to the neutral state (reduction) the negative charges of the immobile counter ions are compensated by that mobile positively charged counterions from the

electrolyte when entering the bulk (Figure 2.1.b).⁵⁴ The volume changing the property of CPs make them also attractive to gain mechanical stimulation of cells.⁵⁵

Figure 2.1: Volume variations during redox reactions: a) For conducting polymer with mobile doping ions: swelling during oxidation and shrinking during reduction, b) for conducting polymer with immobile doping ions: shrinking during oxidation and swelling during reduction.

2.1.3 Surface Switches

Upon redox switching, CPs cannot only switch their properties of the bulk but also those of the outermost layer, i.e along with the very interface of the CP film facing the electrolyte. By switching the redox state of the CP, for instance, the wettability at the surface of the polymer film is controlled.⁵⁶ Wettability, or surface energy is known to impact the adhesion and proliferation of cells. So, by switching the surface properties of a CP, cells can be cultured and grown on

the film finally reaching different tissue states. This allows for the in vitro control of the shape and growth of cells,⁵⁷ cell migration,⁵⁸ and cell adhesion and density.⁵⁹ The cells can change their concentration and their orientation following and adapting to the dynamic properties of the surface switches, controlled by the redox process. Additionally, matrix proteins are crucial in this process and dictate the adhesion and proliferation processes of cells and the orientation and configuration of these proteins can be electronically controlled via the surface switches.⁶⁰

2.1.4 Drug Delivery Systems

Another important property of CPs is its ability to electrochemically control the local release of therapeutic substances. CP electrodes are used for conversion between electronic and ionic signals as a result of the combination of electronic and ionic transport. CPs have been explored for drug delivery applications by incorporating drugs as a dopant ion or embedded molecule into the CP bulk.³⁰ During the de-doping process, the drug is released (Figure 2.2).

The drug delivery process relies on the diffusion process, where the redox state of the CP controls the diffusion rate. For higher spatial and temporal delivery control, also the migration of the actual molecular compound should be added to the delivery mechanism.

The Organic Electronic Ion Pump can be used for high-resolution delivery using polarizable CP electrodes that can control the drift, via electrophoresis, of the charged substances through an ion exchange membrane (Chapter 4).

Figure 2.2: Conductive Polymer drug delivery process. The drug is released by switching the redox state of CP.

2.2 Iontophoresis

Iontophoresis is a mechanism to deliver drugs trans dermally by applying a potential on the skin.^{61,62} Drug molecules are transported through the skin by electrophoresis and electroosmosis and the electric field can increase the permeability of the skin.^{63,64} The transport is measured in units of chemical flux, ($\mu\text{mol}/(\text{cm}^2 \text{ hour})$). Iontophoresis has several experimental, therapeutic and diagnostic applications. Small ions and neurotransmitters can be delivered locally, close to neurons through micro-electrophoretic techniques, including micro-iontophoresis (miniaturized device).⁶⁵

2.2.1 Electrophoresis

Electrophoresis is an electrokinetic process that describes the migration of charged molecules and their separation in a fluid under the influence of an electric field. The system contains two electrodes of opposite charge connected by a conducting electrolyte. The migration of charged molecules depends for instance on pH, the viscosity and the pore size of the medium through which the ions are moving.⁶⁶ The magnitude of migration and level of separation of different charged molecules depends on the applied electric field their mobility, respectively. Electrophoresis is commonly used in biotechnology to separate protein molecules or DNA and can be released through several different procedures depending on the type and size of the molecules.

2.2.2 Osmotic Pumps

Osmotic pumps contain a core carrying an osmotic agent and a target molecule or drug stored in a semi-permeable membrane that absorbs water from the surrounding medium (Figure 2.3). This membrane is permeable to water but impermeable to the drug or molecule. The delivery of the active agent is controlled by the water influx across the membrane. The device generates an osmotic pressure difference between the core, including the osmotic agent, and the surrounding medium. The high osmotic pressure in the core causes water to flow into the pump through the semi-permeable membrane. The

water influx into the pump compresses the reservoir, resulting in a net delivery of the drug through an orifice of the device. The diffusion rate of the solute is minimized by the size and format of the orifice in the device. The small size of the orifice also prevents the generation of hydrostatic pressure and then also protects against decreased osmotic pressure and a change in the volume of the device. The drug release rate is remained constant by that the volume delivered is equal to the volume of the osmotic water uptake.⁶⁷

Cellulose acetate is often used as the semipermeable membrane in osmotic pumps, especially in those based on tablets for controlled drug release. Ethylcellulose is another hydrophobic material commonly used as the membrane material, tablet binder and matrix former in microcapsules and microspheres for controlled release dosage forms.⁶⁸

Figure 2.3: Illustration of an Osmotic Pump for drug delivery applications. Image courtesy of Alzet Osmotic Pumps.

2.2.3 Microfluidic Systems

Microfluidic systems offer several advantages in various biotechnology processes and applications since these techniques can be applied on a scale at which osmotic delivery, electrophoretic transport, and surface interactions occur.⁶⁹

Some of the advantages of microfluidics relate to their compact size, disposable nature, increased utility and when there is a prerequisite for reduced concentrations of the sample reagents. Microfluidic systems are often

used for precise control and manipulation of liquid delivery through micrometre-sized channels. Another application of microfluidic systems is the control over the transport of small individual fluid volumes ($< 1 \mu\text{l}$), where capillaries forces are used to drive the transport and complexing of samples.

These applications are commonly performed within a network system of many channels operating by the passive flow. Specific microfluidic systems have been applied to DNA,⁷⁰ and proteins,⁷¹ analysis methods. Active microfluidics is referred to as the controlled manipulation of a fluid by using active (micro-) components such as micropumps or microvalves. Micropumps are utilized to sample and deliver drug doses close to a defined delivery point, by for instance applying a low potential or a force. The drugs are electrophoretically pumped outward to the target through small holes coated with an ion bridge material.⁷² Microvalves are often used to control the flow direction or the mode of transport of pumped liquids.

3

Ion Transport & Ion Exchange Membranes

3.1 Electrolytes

An electrolyte is electrically conducting since it contains dissolved mobile ions, i.e. positive cations and negative anions. Depending on the degree of dissociation, electrolytes are considered strong, in which ions are completely dissociated, or weak, in which ions are partially dissociated. Each ion has one or multiple charges and the number of positive and negative charges in the electrolyte must be equal to maintain electroneutrality.

The electrolyte ions can be metal ions, more complex organic molecules (drugs or neurotransmitters) or polymer chains containing a high concentration of repetitive charges (polyelectrolytes) (Figure 3.1). A polyelectrolyte with a high concentration of fixed positive charges is called polycation and with negative charges is called polyanion.

Figure 3.1: An electrolyte solution with dissolved ions (left), a polyelectrolyte with fixed negative charges (right).

3.2 Mechanisms of Ion Transport across Membranes

Ion transport in an electrolyte is the directional movement of ions or molecules inside the electrolyte. This can occur through transport across a membrane, passively along concentration gradients (diffusion), active transport in an electric field (migration) and by the movement of fluid (convection) (Figure 3.2).

Figure 3.2: Models of ion transport in an electrolyte: Passive transport along a concentration gradient by diffusion (left), active transport in an electric field by migration (middle) and movement of dissolved ions in a fluid by convention (right).

Diffusion is the net movement of ions or molecules from a higher to a lower concentration region. This process is driven by a gradient in the chemical potential of the diffusing species (from high chemical potential to lower one). The diffusion process is described by Fick's laws^{73,74} (Eqn. 1) for electrically neutral ions in ideal mixtures. The diffusion model expresses the diffusion flux (J) ($\text{mol m}^{-2}\text{s}^{-1}$) through concentration (c) and diffusion coefficient (D) (m^2s^{-1}), a property which defines the ability of a molecule to transport in a solvent.

$$J = -D\nabla c \quad (1)$$

The diffusion coefficient indicates the quantity of a diluted ion that is diffusing through a solvent. It is the magnitude of the molar flux through a surface per unit concentration gradient.

Ions and molecules can be transported in an electrolyte under an electric field by migration. Transportation of ions by migration can create a concentration gradient resulting in a diffusion process as well. The ability of the ions to transport through an electrolyte in response to an electric field is called mobility (μ) and is related to diffusion coefficient (D) according to Einstein relation:

$$D = \mu k_B T \quad (2)$$

where k_B is Boltzmann's constant and T is the absolute temperature.

Similarly, diffusion of different mobility charged ions induces an electric field in the electrolyte, resulting in a migration process. The diffusing ions are transported in the electrolyte by electrostatic forces and by diffusion due to the ionic concentration gradient.^{75,76} Therefore (J) should be properly modified to include those effects. Thus, it is described by the Nernst-Planck equation where describes systems with coupled diffusion and migration:

$$J = -D_i \nabla c_i + z_i D_i c_i f \nabla \varphi \quad (3)$$

where ∇c is the concentration gradient, D_i is the ionic diffusion coefficient, z_i is the charge number, φ is the electrical potential and $f = F/RT$ is the fraction of the ideal gas constant R with value $8.3144 \text{ J mol}^{-1} \text{ K}^{-1}$ and the temperature T by the Faraday constant with the value of 96485 C mol^{-1} . The first part of the equation describes the diffusion contribution to the ion transport and the second part, the migration contribution to the ion transport. The Nernst-Planck equation is valid for dilute electrolytes in which ions do not interact with each other but are in practice often applied to concentrated electrolyte systems as well.

Ions and molecules can be transported in an electrolyte along with the solvent by convection. This process can be achieved via stirring of the liquid or because of a density gradient (arising from temperature differences, concentration

differences).⁷⁷ A viscous polymer gel can prevent the movement of fluid, thereby avoiding convection.

Poisson equation (Eqn.4) describes the ion transport in an electrolyte and can indicate the electric potential (φ) for a given charge distribution. Combining the Nernst-Planck and Poisson equation, we can get a new equation for the electrostatic potential. In the case that the charge density is zero, the electric field is constant, and the gradient of the electric potential is constant.

$$-\nabla(\varepsilon \nabla \varphi) = \sum_i z_i e c_i \quad (4)$$

where ε is the permittivity of a medium, c is the ion concentration, z is the valence of ionic species, e is the elementary charge and φ is the electric potential.

3.3 Ion Transport Numbers in an Electrolyte

An electrolyte contains two or more types of ions and they contribute differently to the conductivity of the electrolyte depending on their mobility (diffusion coefficient).⁷⁸ The ion transport number (t_i) is the fraction of the total electrical current (I_{tot}) due to transport of a specific ionic species (i) (Eqn. 5).

$$t_i = \frac{I_i}{I_{tot}} \quad (5)$$

In the case of electrolytes of ions with nearly equal diffusion coefficients ($D_{K^+} \approx D_{Cl^-} = 2.0 \times 10^{-5} \text{ cm}^2/\text{s}$),⁷⁹ e.g. KCl, the transport numbers for both the cation and the anion are approximately $t_{\pm} = 0.5$ (the sum of transport numbers for different ions in an electrolyte always equals unity). In the case of electrolytes with ions of different diffusion coefficients, for example, HCl (aq), more than half of the current is carried by the positively charges H^+ because of the diffusion coefficient of H^+ ($D_{H^+} = 9.3 \times 10^{-5} \text{ cm}^2/\text{s}$) is significantly larger than

Cl^- ($D_{\text{Cl}^+} = 2.0 \times 10^{-5} \text{ cm}^2/\text{s}$) and the transport number of H^+ is higher than for Cl^- (Eqn. 6).⁸⁰

$$t_{\text{H}^+} = \frac{D_{\text{H}^+}}{D_{\text{H}^+} + D_{\text{Cl}^-}} \quad t_{\text{Cl}^-} = \frac{D_{\text{Cl}^-}}{D_{\text{H}^+} + D_{\text{Cl}^-}} \quad (6)$$

3.4 Ion Exchange Membranes

Ion exchange membranes (IEMs) are cross-linked polyelectrolytes and often used to separate two different electrolytes.⁸¹ IEM with positive and negative fixed charges does not present any selectivity, allowing all the ion species in the electrolytes to be transported through it.

An IEM with a high concentration of fixed positive charges is called anion exchange membrane (AEM) and it is electrically conductive allowing the transport of certain dissolved negatively charged ions while blocking positively charged ions. An IEM with a high concentration of fixed negatively charged ions is called cation exchange membrane (CEM). CEMs and AEMs are analogous to p- and n-type of semiconductors conducting either positive or negative charge carriers respectively. A CEM repels negatively charged ions while attracting positively ones.⁸²

Mobile ions with the same charge as the fixed charges of an IEM are referred to as coions while ions of opposite charges are referred to as counterions. In most cases, the polyelectrolyte chains of CEM contain sulfonates while the polyacrylate chains of AEM contain amines.⁸³ The fixed charges in the membrane are compensated by counterions in order to obey local electroneutrality and coions are repelled. By applying a potential difference across the membrane, the counter ions are transported from one electrolyte to another through the IEM. At the same time, the high density of fixed charges in the membrane repels the coions from entering the membrane (Figure 3.3).

The ionic transport through the IEM is a combination of migration, controlled by an electric field, and diffusion along a concentration gradient. The ionic

current equals the electronic current produced by closed circuit and is carried by the mobile ions.

Figure 3.3: Ion transport through a) neutral membrane, an b) AEM and a c) CEM. AEM and CEM hinder respective co-ions from entering the membrane while allowing counterions to transport through the membrane.

3.4.1 Desirable Properties of Ion Exchange Membranes

Properties of IEM are determined from the density of fixed charges in the membrane and the hydrophobic or hydrophilic character of the matrix polymer. An IEM should present high permselectivity (see below), low electrical resistance, good mechanical stability, and high chemical and thermal stability.

3.4.2 Selectivity and Permselectivity of Ion Exchange Membrane

Selectivity is the membrane capability to separate different ions from each other. The selectivity of an IEM depends on the concentration of fixed charges,

the concentration of the electrolytes, and the ionic current through the membrane. It also depends on the size, the mobility and the configuration of the transported ions and the chemical and geometric structure of the IEM material.³⁶

Organic polymer IEM materials are excellent conductors with effective selectivity for small ions such as H^+ , K^+ , Na^+ , Cl^- and for more complex biomolecules such as γ -aminobutyric acid (GABA), glutamate (Glu), and acetylcholine (AChCl). Permselectivity (S) of an IEM, describes the tendency of the membrane to pass ionic current by counterions.^{84,85}

The permselectivity can be calculated as the fraction difference between the transport number of counterions in the membrane and the transport number of counterions in the electrolyte over the transport number of coions in the electrolyte (Eqn. 7)⁸⁶

$$S = \frac{t_{counter,membrane} - t_{counter,electrolyte}}{t_{co,electrolyte}} \quad (7)$$

An ideal permselective IEM would be permeable for counterions only. The permselectivity is considered as an ideal and approaches 1 when the transport number of counterions in the membrane is higher than the electrolyte and all the ionic current is carried by counterions. In the case that the concentration of the counterions in the electrolytes is much higher than the concentration of the fixed charges in the membrane channel the permselectivity approaches zero.

The permselectivity to cations or anions does not distinguish specific cationic or anionic species though the CEM or AEM and their contribution to the total ion flow depends on the ions's mobility (or equivalently, their diffusion coefficients) and their concentration. The diffusion coefficients influence on ion transport can be problematic, since small ions such as H^+ in the electrolyte have higher mobility than any other positively charged biomolecules in the electrolyte, such as drugs or neurotransmitters molecules. Cation

transportation through a CEM can, therefore, be dominated by H^+ transportation, and equivalently that anion transportation through an AEM can be dominated by OH^- .

3.5 Electric Potentials across Ion Exchange Membranes

The concentration of fixed charges in an IEM creates a potential difference between the membrane and the surrounding electrolytes. The potential difference depends on the concentration of fixed charges in the membrane and the concentration of electrolytes (Donnan Potential) and the mobility of the ions (Junction Potential).

3.5.1 Donnan Potential

When an IEM is in contact with an electrolyte, a concentration gradient for counter ions and coions is formed between the membrane and the electrolytes. The concentration of the counterions in the membrane is higher than in the electrolyte, while the concentration of coions is higher in the electrolyte than in the membrane.⁸⁷ The counter ions move away from the membrane to the electrolyte by diffusion while the coions move to the membrane (Figure 3.5.a).

In the case of an CEM, the transport of the ions by diffusion results in an excess positive charge proximately located outside of the membrane and a negative charge proximately located inside the membrane and reverse in the case of a AEM. This movement creates an electric field (E) at the electrolyte-membrane interface opposite to diffusion process (keep the coions in the electrolyte and the counterions in the membrane). The potential difference between the electrolyte and the membrane is called Donnan potential (φ_D) and is described by the Eqn.8. The produced potential between two electrolytes separated by a membrane is referred to as membrane potential.

$$\varphi_D = \frac{RT}{zF} \ln \frac{C^s}{C_{cou}} \quad (8)$$

where z is the charge, C^s is the electrolyte concentration of the counterions and C_{cou} is the concentration of the fixed charges in the membrane.

The Donnan potential depends on the concentration of electrolyte and of the fixed charges in the membrane and it is negative for CEMs (cations are attracted to the membrane while anions are repelled) and positive for AEMs (anions are attracted to the membrane while cations are repelled)⁸⁸ (Figure 3.5.b). The Donnan exclusion is effective if the concentration of the electrolyte is lower than the concentration of fixed charges in the membrane (attract counter ions in the membrane and repel coions from the membrane).

3.5.2 Junction Potential

Ions diffuse from higher concentration electrolyte to lower one under a concentration gradient.⁸⁸ Under zero-current conditions, the flux of cations and anions should be equal to maintain electroneutrality in the system. However, higher mobility ions diffuse faster than lower mobility ones. These diffusion differences cause a potential difference along the concentration gradient, that opposes the diffusion of the high mobility ions and speeds up diffusion of the low mobility one (Figure 3.5.c).⁸⁹ This potential is referred to as Junction or diffusion potential.

Figure 3.5: Concentration of ions, the electric field, and the electric potentials across a membrane. a) Donnan potential across an electrolyte - CEM system, b) Donnan potential across an electrolyte - AEM system. The electrolyte concentration is polarized across the membrane due to diffusional forces. This results in an excess of positive charges outside the membrane and an excess of negative charge inside the membrane for CEM and opposite for AEM. The potential in the CEM is lower than the potential in the electrolytes since cations are entering the membrane while anions are repelled, and it is higher in the AEM respectively. The concentration polarization across the membrane creates an electric field at the interface between the electrolytes and the membrane. c) Junction potential across an electrolyte - non-charged membrane system. Ions move from a higher concentration electrolyte to a lower one under a concentration gradient. A potential difference along the membrane is created due to the ion diffusion differences.

3.5.3 Concentration Polarization

When a potential is applied across an IEM, counterions migrate across the membrane and accumulate at the release side, which results in a sharp concentration gradient in this area compared to the bulk of the electrolyte. In turn, the electrolyte concentration decreases at the vicinity of the feeding side of the membrane where the counterions enter the membrane, as the electric field forces the counterions into and the coions away from this side of the membrane. At the membrane release side, counterions escape and the coions migrate towards the membrane interface, causes an increasing concentration of the electrolyte at this membrane side. The resulted concentration gradient across the membrane creates a concentration polarization at the sides of the membrane (Figure 3.6.1).⁹⁰

Concentration polarization refers to the emergent phenomena of concentration gradients at the interface between the IEM and the bulk electrolyte as an electric current is applied through the system. The rate of transportation of ions depends on the value of the electric current applied through the membrane.

3.5.4 Limiting Current

Concentration polarization in the classical theory says that the ionic concentration at the source membrane interface drops and approaches zero as the current density increases. Meanwhile, the concentration increases on the opposite side due to the membrane selectivity.

According to Ohm's law, the resulting electric (ionic) current (I) increases linearly with voltage (V) at low voltages, until the concentration drops to zero at the inlet interface of the membrane due to concentration polarization. At this point, the limiting current of the system is reached.^{91,92} The resistance of the system increases and a space-charge region (SCR) is formed. The current that occurs in the system is referred to as limiting current (I_{lim}). Increasing the levels above the limiting current ($i > i_{lim}$, over limiting current),⁹⁰ an additional potential loss is introduced.⁹³ The electric field across the membrane (at the

membrane inlet interface) increases sharply due to the ion depletion at the membrane inlet and causes electric field- enhanced water splitting.

Water splitting mechanism generates protons (H^+) and hydroxide (OH^-) ions at the inlet interface.^{90,94–96} This results in a higher current density in the system. In Figure 2.6.b an archetypical ionic current-voltage (I-V) curve is presented for a CEM with the three characteristic regions in the I-V curve. The first linear region (i) specified by Ohm law for an electrolyte and membrane resistivity values. The second region (ii) is dominated by a plateau originating from the reduction of counterions concentration at the source membrane interface. In the third region (iii), the linear increase in the I-V characteristics, above the limiting current, and it is referred to as over-limiting current.^{91,97–99}

Electrically induced convection, electroconvection, electroosmotic convection and/or water splitting at the inlet of the membrane cause the over-limiting current region.^{100,101} Inhomogeneities of the membrane results in an irregular electric field that interacts with the extended space charge. This is built up as a result of an excess of counterions adjacent to the membrane which is established above the limiting current density.¹⁰² The limiting current density is determined as the intersection of the tangents of the curve of regions (i) and (ii) (Figure 3.6.c).

Figure 3.6: a) Concentration polarization across an IEM for different current levels, b) Typical current-voltage curve of the three regions related to limiting current ((i) non limiting current linear region, (ii) limiting current region, (iii) over limiting current region with potential water splitting), c) Correlation between each characteristic region with a schematic illustration of electrolyte – CEM system.

3.5.5 Water Splitting Production

Water splitting (or water dissociation) is a phenomenon in which water molecules are dissociated into H^+ and OH^- . For an electrolyte – IEM system, above the i_{lim} level, a space charge region is produced at the feeding side results H^+ and OH^- ions generation at long the inlet interface between the membrane and the electrolyte. Then, H^+ and OH^- are transported into the CEM or AEM respectively.⁹⁰ This generation has as a result of high current and changes in pH at the electrolyte – membrane.¹⁰³

4

Iontronics

Over the last decade, a new technology based on sophisticated control of ions as signal carriers have emerged to bridge organic electronics and biological systems. This new technology is referred to as “iontronics” and it can be used for many applications, including flexible electronics and healthcare-related devices.¹⁰⁴

Iontronics is considered as the science and technology of electronic properties/functions controlled by ionic motion and arrangement.⁹⁰ The development of iontronic components can affect precise ion transport. In many organic electronic components, such as organic electrochemical transistors (OECTs),¹⁰⁵ electrolyte gated organic field-effect transistors (EGOFETs),^{15,106} and iontronic delivery devices,^{22,107–110} ions play a fundamental role and the device functionality is defined by the coupling of ionic and electrical signals in conductive polymers and (poly)electrolytes. The most basic iontronic component is the organic electronic ion pump (OEIP) (Figure 4.1), described in detail below. OEIPs transport ions or biomolecules electrophoretically from a source reservoir, through an IEM, and to a target system such as cells or tissue. They are characterized by a controlled release of charged ions or biomolecules at specific times and specific locations, resulting in a chemical gradient. OEIPs can be combined into iontronic circuits for addressing and signal processing.³⁶

Compared to traditional electronics, iontronic circuits use ions as the majority charge carriers rather than electrons, and they can generate complex ionic (chemical) signal patterns at high spatiotemporal resolution and biochemical specificity.³⁸ Iontronic drug delivery devices offer several advantages in comparison with other delivery technologies such as microfluidic and microelectromechanical system (MEMS) based micropumps,¹¹¹ and iontophoresis.^{112,113} Specifically, they provide a means of simultaneously

achieving high delivery precision at low doses, negligible liquid transportation, and control over delivered amounts thus limiting potential side effect.³⁶

Figure 4.1: Schematic illustration of a planar organic electronic ion pump (top) and a typical potential profile of an OEIP with applied potential (bottom).

4.1 Devices developed in the thesis

In the last ten years, OEIPs have been used to trigger cell signalling in vitro,^{107,114} to control epileptiform activity in brain slice models,^{115–117} to effect sensory function in vivo,⁴⁶ to suppress pain sensation in awake animals,¹¹⁰ and to modulate plant growth.¹¹⁸ The first OEIP device was made by Isaksson *et al* in 2007 to study neurological signalling in vitro via the delivery of positive metal ions.¹⁰⁷ In this thesis, various OEIPs are developed and used for investigating the ability of differently charged ions (small ions, neurotransmitters, and drugs) to be delivered, focusing on the fundamental understanding of ion transport and device physics.

In Paper I, **planar OEIPs on glass substrates** were developed and used to investigate the role of pH of a delivered biomolecule in electrophoretic transportation in iontronic devices. The results provided a functional method of optimization for future applications.

In Paper II, we demonstrated **OEIPs with Pd contacts patterned along the IEM** improving the delivery rate and the selectivity of the therapeutic substances of interest, while preventing undesired pH changes in a target such as biological tissue.

In Paper III, we miniaturized OEIPs by incorporating them into **glass capillary fibers** and we demonstrated various engineering solutions to optimize the transportation and the delivery of different therapeutic substances at low concentrations.

In Paper IV, we successfully investigated the treatment of inflammatory monocytes by delivering anti-inflammatory drugs using **capillary fiber OEIPs**.

4.2 Organic Electronic Ion Pumps (OEIPs) – Structure and Function

The OEIP is an electrophoretic delivery device with high spatiotemporal delivery resolution and it operates as an iontronic resistor.^{108,110,119} The main function of an iontronic resistor in a circuit is to control the flow of ionic current to other components following Ohm's law and behaving exactly like an electronic resistor. The resistance depends on the ionic concentration, the type of ions and the geometry of the area in which ion current is carried.

The basic OEIP comprise a source electrolyte containing the ions to be delivered (neurotransmitters such as GABA⁺, ACh⁺, drugs such as SA⁻ or small ions such as H⁺); an ion channel, like a salt bridge going from source to target; a target electrolyte (to which ions are delivered) which could be, for example, a cell culture or tissue slice; and cathode and anode electrodes (for example, Ag/AgCl paste electrodes) (Figure 4.1).

It can be used to deliver small ions, drugs, and neurotransmitters directly where they are needed on-demand, thereby limiting potential drug side effects. These active substances can be delivered to tissue slices and cell culture stimulating or reducing cell activity locally. The OEIP is based on selective transport, or "pumping", of charged species (small ions, drugs, neurotransmitter) through a cation- or anion-exchange membrane (CEM or AEM), resulting in high dosage precision; in principle, one electron corresponds to one delivered monovalent ion. Unlike analogous microfluidics-based techniques, OEIPs exhibit negligible liquid flow.

CEMs and AEMs are characterized by a high concentration of fixed negative or positive charge, respectively. The permselectivity is ideal in the case that the concentrations in the adjacent electrolytes are lower than the fixed concentration of the membrane (referred to as Donnan exclusion).⁸⁸ When a potential difference is applied between the two electrolytes, the counterions of the source electrolytes are able to selectively migrate across the IEM while the co-ions are blocked from drifting in the opposite direction. Electrochemical reactions of Ag/AgCl paste electrodes in the source/target electrolytes drive the current through the OEIP according to Eqn. 9.

In the case of an AEM, for each chloride that is introduced in the source electrolyte from the AgCl electrode reaction, one negative charge will leave the source electrolyte and will travel through the positively charged membrane to the target electrolyte, in order for charge neutrality to be maintained. In the case of a CEM, for each chloride that is introduced in the AgCl electrode, one positive charge will leave the source electrolyte respectively based on the different concentrations of the positive charge vs $[\text{Cl}^-]$.

The ionic current in OEIPs relies on the applied electric field. The permselectivity of the AEM or CEM does not distinguish specific counterion species, leading for example, to co-transport of H^+ and GABA^+ . The relative contribution to the total ion flow through the membrane channel relies on the various species' size and mobility (μ) through the membrane or equivalently, their diffusion coefficients (D) according to the Einstein relation (Eqn. 10) in kinetic theory:

$$D = \mu k_B T \quad (10)$$

where k_B is Boltzmann's constant and T is the absolute temperature.

The actual selectivity of the OEIP is determined by chemically quantifying (for example, using ELISA, enzyme-linked immunosorbent assay) the delivered number of ions in the target electrolyte after a measured amount of charge is recorded in the electrical circuit.

4.2.1 OEIPs Applications

4.2.1.a *In Vitro*

OEIPs have been used to stimulate cultured neuronal cells and study their neurological signals *in vitro* by delivery of small metal ions such as K^+ , Na^+ and Ca^{2+} ,¹⁰⁷ and neurotransmitters such as acetylcholine (ACh).¹⁰⁸ These devices relied on cell depolarization by delivering small ions and biomolecules which activate and trigger Ca^{2+} signaling.¹²⁰ Succeeding here, wider OEIPs channel was developed to transport more complex biomolecules such as GABA and Glu and the amino acid and excitatory neurotransmitter aspartate.⁴⁶ Further, the dynamic delivery of the neurotransmitter ACh was performed using nonlinear “ion transistors” to stimulate SH-SY5Y cells.¹²¹ OEIPs have been used to induce pH gradients and pH oscillations in the target electrolyte by controlled delivery of H^+ ,¹¹⁹ to provoke amyloid aggregation of polymer models by delivering H^+ and Na^+ .¹¹⁴ Later, OEIPs were also used on for H^+ delivery to investigate amyloid aggregation in actual A β proteins associated with Alzheimer’s disease.¹²²

4.2.1.b *In Vivo*

Over the years, OEIPs have been adapted for *in vivo* implantable applications. The first of these OEIPs were used to stimulate the guinea pig cochlea via glutamate (Glu) delivery.⁴⁶ Delivering Glu into the cochlea through the round window membrane, a strong auditory response was observed. This experiment investigated the ability of OEIPs to target specific cells, as only cells with Glu receptors were affected. The next step of implantable OEIPs was used in the first organic electronic delivery device for the treatment of neuropathic pain in an animal model. These devices were implanted onto the spinal cord of rats and the inhibitory neurotransmitter GABA was locally delivered, resulting in a significant decrease in pain response with a low dosage and no observable side effects.¹¹⁰ For localized delivery of drugs to the brain, microfluidic ion pumps (μ FIPs) were used. These devices show the ability of K^+ to be delivered to the cortex of a rat, resulting in hyperexcitability within seconds after the device turned on.⁷²

4.2.1.c In *Plantae*

More recently, an implantable OEIP was used to demonstrate the first organic electronic delivery devices to plants. Changing the geometry of the OEIP, biologically relevant quantities of the plant hormone auxin were delivered to the root tips of living *Arabidopsis thaliana* seedlings.¹¹⁸ This auxin delivery via fiber capillaries OEIPs induced a physiological response, observed as the slowing of the root's growth rate.

4.2.2 Advantages of Organic Electronic Ion Pumps

OEIP-based technology offers several devices and functionalities for bioelectronic applications. Using OEIPs, electronic control over the delivery of precise doses of small ions and therapeutic substances such as drugs and neurotransmitters to micrometre-scale locations, without convection, at high temporal resolution can be achieved. This technology induces the delivery of an active substance without liquid transport while limiting potential side effects associated with other delivery techniques (in particular, systemic delivery). In the past, many delivery techniques were ineffective since chemicals are dissolved and could be delivered along with their aqueous solvent making the dose control difficult. The liquid transport could also perturb the target environment and increase the local pressure in proximity to the target tissues or cells.

4.2.3 Limitation of planar OEIPs

Originally, OEIPs were only built on two-dimensional substrates. This technology is based on photolithographic techniques for patterning active, followed by passive, areas. Nonetheless, for an implantation context, when the delivery should occur locally within a cell or tissue, scale and rigidity limitations along are stiffened against on this design resulting in issues related to invasiveness (planar substrates/geometry and flexibility).

Additionally, in planar devices, the IEM developed via thin-film processing, can be tailored based on an intrinsic ionic resistivity which depends on its geometry

(length/width ratio) and on the mobility of the ions that are being transported.³⁶ Planar devices with narrow channels present a higher resistance in comparison with wider channels for large ions transportation given the same length/width ratio. The high resistance is related to the tendency of narrow channels to present less swelling as compared to wider ones providing less water uptake inside the IEM that is necessary to promote high ionic conductivity.¹¹⁰

4.3 Fiber Capillaries Organic Electronic Ion Pumps

To overcome the limitations related to molecules size, to design's device and to implantation applications, we developed OEIPs devices based on glass capillary fibers that are filled with a polyelectrolyte (IEM). The fibers are assembled into a polyolefin heat-shrink tube work as a source reservoir (Figure 4.2). This design provides several advantages for use with implantable devices. These devices also serve as both encapsulation and substrate and can easily be implanted into proximity to targeted cells, tissues and organs. Fiber capillary OEIPs serve a larger ion-transport cross-section make them the best candidate for large biomolecules transport.

Figure 4.2: Schematic illustration of fiber capillary OEIPs (left), a photograph of fiber capillary OEIP (right).

5

Fabrication & Characterization

5.1 Device Fabrication

Organic electron-ion pump (OEIP) devices are fabricated based on cationic or anionic exchange membranes (CEMs or AEMs) using microfabrication techniques. The fabrication techniques are chosen according to the intended device design. Traditionally, bioelectronics demonstrations of OEIP technology have required photolithographic techniques on planar substrates to sequentially pattern active and passive areas.

These techniques involve spin coating to deposit the selective membrane material, thermal evaporation, photolithography to pattern the electrodes and the CEM or AEM channel, and reactive etching. In the case of OEIP devices based on glass capillary fibers, the fabrication techniques are restricted to filling the glass capillary with an anionic or cationic polyelectrolyte using flushing techniques and UV exposure for the polymerization of the monomer with the photoinitiator.

OEIP fabrication processes take place in a cleanroom environment to limit the atmospheric particles and the dust since the number of particles per volume in the cleanroom is controlled and kept under certain limits.

5.2 Fabrication Techniques of Planar OEIPs

5.2.1 Preparation of PSS-co-MA/PEG

The OEIPs presented in Papers I and II were fabricated on glass substrates using a custom-developed polyanion membrane material, poly(4-styrenesulfonic acid-co-maleic acid) (PSS-co-MA), where the maleic acid groups can form ester bonds to a polyalcohol. This material gives stable films on glass and it provides enhanced compatibility with in vitro cell studies. The PSS-co-MA is mixed with polyethylene glycol (PEG) for cross-linking and it forms a membrane that will not dissolve in solution. It is transformed from Na^+ form to H^+ form ($\text{PSS}^-:\text{Na}^+ \text{-co-MA}^-:\text{Na}^+ \rightarrow \text{PSS}^-:\text{H}^+ \text{-co-MA}^-:\text{H}^+$) by dialysis in HCl(aq) since the proton form is needed for cross-linking.¹²³

5.2.2 Spin Coating

Spin coating is one of the most common techniques to deposit materials from a liquid solution onto planar substrates.¹²⁴ We used this technique to deposit polymer CEMs or AEMs and the photoresist layers on planar devices. This technique offers several advantages over other deposition techniques since it is repeatable, and it can easily and quickly produce a uniform and homogeneous thin film between 10 nm and 100 μm thick.

As a first step, a liquid, containing the material to be deposited in a solvent, is applied onto the substrate and then the substrate is rotated at a slow spin rate (500 rpm) for a couple of seconds (3-5 sec). In this “spreading” mode, most of the liquid is flung off the side as the substrate is fully covered. As a second step, the rotation speed is increased (1000-6000 rpm) for 10-60 sec and the solvent starts evaporating leaving the desired material on the substrate (Figure 5.1).

The thickness of the film depends on the material, the solvent, the concentration of deposited liquid and the spinning speeds that are used. Typically, lower concentration liquids and higher spinning speeds result in thinner films.¹²⁵

Figure 5.1: Deposition of a thin film by spin coating.

5.2.3 Thermal Evaporation

Evaporation is used in microfabrication to deposit thin films under vacuum. The vacuum allows vapor particles to travel directly to the target substrate, where they condense back to a solid-state and form a thin film. Low pressures are used, about 10^{-5} Torr, to avoid reaction between the vapor and atmosphere. At low pressures, the mean free path of vapor atoms is in the same order as the vacuum chamber dimensions, so these particles travel in straight lines from the evaporation source towards the substrate. The source material (the material to be deposited) is heated under vacuum. The evaporated metal particles are spread and deposited directly to the target substrate, where they condense to a solid-state and create a thin film. In the thermal method, metal materials (Au, Cr, Ti, etc. in the form of pellets) are placed onto heated evaporators ("boats" capable of withstanding high temperatures). The source material starts subliming and evaporating into a cloud above the source. The evaporated particles are deposited on the target substrate creating a thin film (Figure 5.2).¹²⁶

Figure 5.2: Deposition of a thin film by thermal evaporation.

5.2.4 Photolithography

Photolithography is a microfabrication technique used to pattern regions of thin films.¹²⁷ A light source is used to transfer a designed pattern from a mask to a photosensitive chemical material (photoresist) deposited by spin coating on the substrate. The pattern on the photoresist film is created by exposing it to UV light through the mask (Figure 5.3). This exposure drives a chemical change of the photoresist allowing part of it to be removed by a chemical developer. Only the photoresist part under the transparent parts of the mask are exposed. A positive photoresist is soluble in the developer after light exposure while the negative photoresist is insoluble in the developer since the exposed parts of the photoresist film start cross-linking.

The developing step creates the pattern of the mask, dissolving the exposed or unexposed parts depending on the photoresist. The minimum obtainable size features and the gaps between them define the technique's resolution.¹²⁸ The resolution depends on various considerations such as the thickness of the photoresist and the UV light, leading to feature resolution from nm up to μm . For organic electronics, the resolution limit is typically 1-2 μm .

A mask aligner machine is used in the exposure step to control and correctly align the position of the substrate in relation to the mask, especially when the produced device comprises several photolithographically patterned material layers that must be precisely positioned relative to each other. The optimum alignment of the different layers offers high-resolution patterning. Special alignment marks on the mask, outside the relevant pattern, can control, very precisely, the position of the substrate and the exposure dose. With mask aligner, we can position the different material layers correctly on the substrate for each exposure and produce stacks of the patterned layer.

Figure 5.3: Illustration of photoresist patterning by UV light exposure through a mask in the mask aligner. The positive photoresist is exposed by UV light through a mask in the mask aligner. Vertical dimensions are greatly exaggerated.

5.2.5 Reactive Ion Etching (RIE)

In RIE (“dry etching”), the deposited material under the photoresist is removed by a chemical reaction from a chemical agent in plasma. The photoresist ($1.8\ \mu\text{m}$) is much thicker than the material to be etched ($\sim 500\ \text{nm}$). Even though everything is etched down, the photoresist is only partially etched, while the patterned material is “consumed” down to the substrate (or some lower layer). The plasma is generated by a radio frequency (RF) electromagnetic field under low gas pressure (vacuum chamber) between two metal electrodes.

The substrate is placed on the bottom electrode plate. High energy ionized gas attacks the substrate vertically by an applied bias and reacts chemically to produce anisotropic etching. The electromagnetic field accelerates the ionic species and the substrate to be etched is bombarded by the positive ions. Chemical reactions and bombardment contribute to material etching. Polymers are efficiently etched in an oxidizing O_2 plasma and CF_4 can increase the etch rate or modify the etch chemistry.¹²⁹

5.2.6 Lift-Off

Lift-off is an alternative technique of patterning thin-film material layers.¹³⁰ In this case, a “sacrificial layer” of a positive photoresist is patterned on the substrate followed by the deposition of the material to be patterned. After the material deposition over the whole area of the substrate, the photoresist is washed away with a solvent followed by lift-off of the remaining photoresist in, for example, acetone. The final layer is patterned only in the regions where it had direct contact with the substrate. Lift-off is a common method for patterning metal (Au) electrodes on glass (Figure 5.4).

Figure 5.4: Metal electrodes patterning by a lift-off method.

5.3 Fabrication of Planar OEIPs

All the glass planar substrates were cleaned and rinsed in acetone, isopropanol, and deionized water to remove particles. The next cleaning step comprised treatment with O₂ plasma for 5 minutes (O₂ 400 sccm (standard cubic centimetres per minute) at 250 W) to remove the organic compounds and activate the surface of the substrate, improving the material adhesion to the glass.^{131,132} Activating the glass substrate with O₂ plasma causes hydroxyl groups to be exposed and then they can react with the adhesion promoter (3-glycidoxypyltrimethoxysilane) (GOPS). After the cleaning process, the Au electrodes were deposited by thermal evaporation and Pd electrodes by electrodeposition (in some cases). At this point, the devices comprised substrates with patterned Au electrodes. Subsequently, the polymeric membrane was deposited from liquid solution onto the planar substrates by spin coating. An encapsulation SU-8 layer was spin-coated over the membrane and patterned as the last step. The fabrication of OEIP devices in Papers I and II involve the microfabrication steps presented in Figures 5.5 and 5.6.

5.3.1 Au Electrode Deposition and Patterning

In Paper I, the starting point was the deposition of Au electrodes by thermal evaporation. The photoresist (S1818) was deposited on the top of the Au film by spin coating and then it was exposed and developed. The resulting electrode pattern was achieved using chemical etching. Chemical etching can selectively remove the residual Au material using baths of temperature-regulated etching chemicals. In this manner, the metal electrodes can be patterned with high precision. The residual photoresist was removed using acetone. In Paper II, the starting point was to pattern the photoresist (S1818) followed by the deposition of Au by thermal evaporation. The pattern of the metal contacts was achieved by a lift-off process (sonication in 80% v/v Acetone and 20% v/v IPA for 5 min).

5.3.1.a Palladium Nanoparticle Deposition

In Paper II, an additional photoresist process defined the area of the Pd contacts for Pd nanoparticles (PdNPs) deposition, while the metal interconnects were insulated. PdNO_3 was dissolved in 50 mM nitric acid to give a final concentration of 5 mM Pd. PdNPs were electrochemically deposited (electroplated) onto the Pd contacts using a DC voltage of $V = -0.3$ V vs Ag/AgCl reference electrodes and Pt as a counter electrode with a varied deposition time between 0.1 and 10 s.

The deposition of Pd could be optically observed by the darkening of the contacts where the NPs were successfully deposited. After PdNP deposition, the photoresist was stripped with acetone and the samples were activated with O_2 in order to promote adhesion and binding of GOPS in the glass substrate.

5.3.2 CEM Channel Deposition and Patterning

The substrates were soaked the well-known adhesion promoter 3-glycidoxypropyltrimethoxysilane (GOPS) for 7 minutes.¹²³ GOPS forms silanone bonds to the glass substrate and contains an epoxy group that can react with the polymer material. The polyanion PSS-co-MA/PEG was deposited by spin-coating followed by baking at 110 °C overnight. A thin layer of poly(methyl methacrylate) (PMMA) was spin-coated on top of the PSS-co-MA/PEG film for improved adhesion of the photoresist layer. PMMA protects the polymer from reacting with the photoresist and from damage from the developer.

The photoresist was exposed using a Karl Süss MA6-BA6 Mask Aligner to pattern the photoresist layer to define the CEM channel and developed in Microposit MF319 developer. Reactive ion etching in an oxidizing O_2 plasma (100 sccm) and CF_4 (200 sccm) for 95 s at 150 W was used to remove the deposited material under the photoresist and obtain the patterned PSS-co-MA/PEG CEM channel. The remaining photoresist and PMMA were removed (dissolved) using acetone. Substrates were then soaked in 0.1 M NaCl(aq) for 5 min to ensure that Na^+ remained the dominant counterion. At this point, the

devices comprised the glass substrate with patterned Au electrodes and CEM membrane.

5.3.3 Encapsulation

An encapsulation layer of the negative cross-linking photoresist SU-8 was spin-coated over the CEM channel and patterned. The SU-8 pattern defined hydrophobic confinements for the two electrolytes, source and target, and an encapsulation over the CEM channel. Encapsulation is the last microfabrication step.

Figure 5.5: Fabrication steps for planar OEIPs.

Figure 5.6: Fabrication steps for planar Pd OEIPs.

5.4 Fabrication of Fiber Capillary OEIPs

The fabrication of the fiber capillary OEIPs presented in Papers III and IV involves the microfabrication steps¹³³ presented in Figure 5.7.

5.4.1 Preparation of AMPSA (CEM)

The fiber capillary OEIPs presented in Paper III were fabricated using a polyanion membrane material (that is, a CEM). The acrylate monomer (2-acrylamido-2-methylpropane sulfonic acid) (AMPSA) was mixed with polyethylene glycol diacrylate (PEG-DA, M_w 575, 2 wt%) and a photoinitiator (2-hydroxy-4'-(2-hydroxyethoxy)-2-methyl-propiopheno-ne) (0.5 wt%) that initiates the polymerization of the AMPSA.

5.4.2 Preparation of AETMAC (AEM)

The fiber capillary OEIPs presented in Paper IV were fabricated using a polycation membrane material (that is, an AEM). The acrylate monomer (2-(acryloyloxy)-ethyl trimethylammonium chloride) (AETMAC) was mixed with poly-ethylene glycol diacrylate (PEG-DA, MW 575, 2 wt%) and the same photoinitiator as for the AMPSA CEM material, (2-hydroxy-4'-(2-hydroxyethoxy)-2-methyl-propiophenone) (0.5 wt%) which initiates the polymerization of the AETMAC.

5.4.3 Capillary Fiber Preparation and Filling Process Set-Up

Glass capillary fibers (Polymicro Technologies) were cut by hand to a length of 30 cm using a ceramic cleaving stone. The protective polyimide coating on the surface of the fiber capillary was removed by baking (550 °C for 1 hr) or soaking the capillary in a beaker containing sulfuric acid (concentrated). Above 100 °C, the sulfuric acid removes the polyimide under a slow stirring for 20 min. When the protective coating was removed, the glass capillary was rinsed with DI water. One end of the capillary fiber was assembled to a plastic Luer-lock tip (Nordson EFD) by heat-crimping with tweezers, thus allowing the capillary fiber to be attached to EFD compatible syringes. The syringe reservoir was connected to the nitrogen supply line to give the desired flow rate of the different solvents.

5.4.4 Capillary Fiber Surface Preparation – KOH

The capillary fibers were treated to promote the polyelectrolyte anchoring to the inner surface. In the first process step KOH (2 M) was flushed for 2 h to activate the glass surface with hydroxyl groups. The etching step increased the hydrophilicity of the capillary's inner wall. KOH solution increases the surface silanone concentration because hydroxide ions react with the silanol groups of the silica surface to produce silicate ions.

5.4.5 Capillary Fiber Surface Preparation – Salinization

The next process step was salinization. 3-(trimethoxysilyl) propyl methacrylate (3-TSA, 10 wt% in toluene) was flushed for 1 h followed by drying with nitrogen flushing for 5 min and ethanol flushing for 10 min. Acrylate groups are introduced on the surface of the fused silica capillary and ensure that the polymer is attached covalently to the capillary wall. The silanizing agent reacts with the silanol group on the glass surface and methacrylic groups are expressed on the surface providing hydrophobic characteristics.

5.4.6 AEM/CEM Deposition

The last process step was the filling of fiber capillaries with either the AMPSA (CEM) (Paper III) or the AETMAC (AEM) (Paper IV). The un-polymerized polyelectrolyte solution was flushed by applying pressure to the connected syringe into the capillary fiber until a droplet at the capillary fiber outlet was observed, approximately 20 min (Figure 5.7).

5.4.7 Photopolymerization

The final step was the channel polymerization using a UV lamp (254 nm, 0.2 mW/cm²) for 10 minutes.

5.4.8 Fiber Capillary OEIPs Assembly

The filled and cross-linked capillary fibers were cleaved into 15 mm long sections by a fiber cleaver. The individual fibers were then immediately assembled into a polyolefin heat-shrink tube containing sealant glue, with heat crimping using heated tweezers. The heat shrink tubing served as both a way to handle the capillary-OEIP, as well as an electrolyte reservoir.

Figure 5.7: Fabrication steps for fiber capillary OEIPs.

5.5 Devices Characterization

After fabrication, the OEIPs were characterized by various techniques, described below. The delivery efficiency of the devices was defined as the ratio between the number of intended ions transported into the target (according to chemical quantification by sampling the target electrolyte after delivery) to the number of electrons recorded in the driving circuit (integrated current). In addition, a stereo light microscope was used for initial investigation of the different layers of the device. This method can indicate any non-uniformity or cracks of the polymer film, swelling or delamination of the polymer film and of the encapsulation layer, errors in the pattern alignment, and presence of dust or other particles.

5.5.1 Electrical Characterization

Electrical characterization was performed to evaluate the ability of small ions and drugs to efficiently be delivered and to investigate the function of the devices and characterize the ion transport in materials and devices. We test the OEIPs applying a potential difference between the source and target and

measuring the recorded current. For OEIPs the electric current equals the ionic current.

The OEIPs work as resistors presenting a linear current voltage behaviour that follows the Ohm's law for given electrolyte and membrane resistivity values. The characteristic curve of current versus time is given for a membrane with two district regions. The first linear region is related to channel loading. The second region is characterized by a plateau obtaining a stable current resulting from the ions delivery (Figure 5.8). The time to steady current and the stable current level are dependent on the relative size and abundance of the transported ions. The steady current level is increased when the ions in the source are exchanged to ions with higher mobility.

The OEIPs resistance depends on the size and mobility of the transported ions. The resistance is lower for smaller ions with higher mobility. The current depends on the transport of the counter-ions. Co- ions do not contribute to recorded current. A current variation can be observed as the co-ions in the target electrolyte are exchanged. This is an indication that the co-ions contribute to the recorded current and there is backflow and if that is the case the OEIP doesn't work properly.

In this work, electrical characterization was performed using a Keithley 2602 SourceMeter (Keithley Instruments Inc.) with a custom-designed LabVIEW (National Instruments Corp.) software.

Figure 5.8: Characteristic curve of current versus time for a membrane.

5.5.2 Chemical Quantification

Chemical quantification of the amount of delivered ions and analysis of the ion concentration were performed using an enzyme-linked immunosorbent assay (ELISA). The principle of Elisa is based on the specificity of one antibody for a particular antigen. The antigens of the sample are immobilized on a plate either non- specifically (absorbed to the surface) or specifically (captured by another antibody specific to the same antigen). The next step is the addition of the detection antibody forming a complex with the antigen. The detection antibody is covalently linked to an enzyme or is detected by a secondary antibody that is linked to an enzyme. The last step contains the addition of an enzymatic substrate to produce a visible signal (color change), which indicates the quantity of antigen in the sample.^{134,135} The quantification is achieved by comparing the absorbance of the sample with a standard curve prepared with known standards solution.

In Papers, I, II, the target solutions were collected after GABA delivery, and the amount of delivered molecules were measured using a GABA enzyme-linked immunosorbent assay (ELISA) kit (LDN/ BA E-2500)¹³⁶ measured on a BioTek Synergy H1m plate reader according to the manufacturer's instructions. GABA ELISA is a competitive assay (Figure 5.9). Antibodies are immobilized on the solid phase of the plate and the antigens of the sample are binding forming antibody/antigen complexes. Secondary antibodies, specific to the primary antibodies are added. The secondary antibodies are coupled to the enzyme that elicits a chromogenic or fluorescence signal. Free antigens and antibody/antigen complexes are removed, and the secondary antibody/enzyme is detected by an anti-rabbit IgG-peroxidase conjugate. The reaction is monitored at 450 nm.

In Paper IV, the quantification was performed using a salicylates enzyme-linked immunosorbent assay (ELISA) kit (Neogen).¹³⁷ SA ELISA kit operates based on the competition between the drug in the sample and the drug-enzyme conjugate for a limited number of antibody binding sites following the GABA assay principle. The presence of the drug/enzyme conjugate is recognized by the addition of K-Blue substrate (TMB). The absence of the drug in the sample will result in a darker colour and the presence of it will result in a lighter colour.

Figure 5.9: Competitive Elisa assay.

5.5.3 Chemical Delivery Characterization

In Paper II, the protons entrapment was studied and evaluated including pH indicator in the target electrolyte. Protons are small ions with higher mobility than other ions and they appear faster at the outlet results a colour change at the target electrolyte.

In Paper III, the water-splitting effect was studied monitoring the fiber's inlet and outlet in the presence of a pH indicator using a horizontal spectral microscope system during ions delivery in real-time. Accumulation and transport of OH^- and H^+ at the fiber inlet and outlet respectively results in a colour change confirming the presence of water splitting.

5.5.4 Molecular Dynamic Simulations

In Paper I, the role of GABA's pH-dependent conformational changes were investigated by performing classical molecular dynamics (MD) simulations. For the simulation models we setup the same conditions with the experimental conditions. GABA molecules were solvated in water with a concentration of 0.1 M and mixed with n-propanol, PEG, and PSS at concentrations 8.6 wt%, 0.8 wt%, and 1.78 wt%, respectively according to real GABA concentration in the

source solution and the material membrane composition. Three scenarios were modelled and investigated: i) 100% cationic GABA, ii) mixture of cationic GABA 80% and zwitterionic 20% and iii) mixture of cationic GABA 50% and anionic GABA 50%. Partial charges per atom in each molecule were calculated using *ab initio* density functional theory (DFT) and fit to the electrostatic potential (ESP)¹³⁸ as implemented in the Gaussian software suite.¹³⁹

These simulations pointed to an ideal pH for GABA transportation relating to its configuration and diffusion coefficient. Importantly, these results ascertain the correlation between the simulations and the experimental data, and they are an evidence that the high efficiency at a specific pH is due to the higher ion mobility through the channel as well as to a globular conformation of GABA at this pH.

5.5.5 Finite-Element Modeling of Transport

In Paper III, a finite-element modelling (COMSOL) simulation of the Nernst-Planck-Poisson equations was performed for the inlet of a fiber capillary ion pump to investigate the ion transport process and the effect of limiting current.¹⁴⁰ The inlet was modelled for different ionic species at different concentrations and applied voltages in order to determine the limiting current. The model assumed a homogeneous membrane with a fixed charge concentration. A 2D axisymmetric geometry was used for the model, making it a 2D computational problem.

This model shed light on the effect of limiting current especially for large molecules with lower diffusion coefficient at low concentrations. Thus, the geometry of the membrane can be tuned within different operation windows in order to reach a non-limiting current process.

5.5.6 In Vitro Cell Experiments

In Paper IV, *in vitro* experiments were performed using fiber capillary OEIPs to investigate the anti-inflammatory power of salicylic acid (SA) on monocytes. SA

is the active form of acetylsalicylic acid (ASA). It is a medication, often used to treat pain, fever, and inflammatory diseases since it is classified as a “nonsteroidal anti-inflammatory drug (NSAID)”. This drug is important since it can be used long-term, at low doses, to help prevent heart attacks, strokes, and blood clot formation in people at high risk of developing blood clots.

Isolated monocytes in DMEM cell culture medium supplemented with 10% pooled natural human serum were cultured in a polypropylene Eppendorf tube and placed in a 37 °C incubator. SA was delivered to monocytes and then the cells were stimulated by lipopolysaccharides (LPS) which promote pro-inflammatory cytokines production. The OEIP-mediated prevention of LPS-induced inflammation was indicated by the quantification of cytokines before and after SA delivery using plate reader.

6

Overview of the Publications

6.1 Paper I: pH-Dependence of γ -aminobutyric acid transport

In Paper I, we reported a systematic investigation of the role of pH on ion transport in organic electronic ion pumps (OEIPs). This is important because it lays the theory and framework for optimizing OEIP-based delivery of a significantly wider range of compounds, and thus applications in medical- and biotechnology. To enable the delivery of an assortment of bio-active “ions”, we adjust the pH in the reservoir system such that the transport substance becomes (de)protonated and charged. However, adjusting the pH with additional H^+ or OH^- makes a significant amount of these ions available for concurrent delivery. Thus, the efficiency of transporting the intended ionic species becomes suboptimal and difficult to predict; and no systematic studies of this concurrent transport had been done.

Here we developed a systematic study of the effects of pH on transport efficiency. Specifically, we used OEIPs configured for cation transport of the primary neurotransmitter γ -aminobutyric acid (GABA) (pK_a 4.2). The pH in the reservoir was varied from below to above GABA's pK_a , and the transport was characterized both electrochemically and chemically (with [GABA] ascertained by assay). Molecular dynamics simulations were also performed to elucidate the pH-dependent conformation of GABA and how this could affect transport. All experimental results converged on a specific pH (~ 3) which optimizes the trade-off between the intended GABA transport and concurrent H^+ transport.

These findings and methodology for $GABA^+$ have general implications for all OEIP/iontronic transport. With a general approach for pH optimization, better and more consistent transport efficiency can be achieved. Thus, iontronic

technologies can directly benefit from a more consistent dosage of therapeutic or other bio-active substances, facilitating more wide-ranging applications.

Contribution to this Paper: Developing of fabrication techniques, performing electrical and chemical characterization, analysis and evaluation of the results, and writing the manuscript.

6.1.1 pH Challenge

We indicated that pH 3 is an ideal source pH for efficient GABA transportation. However, the permselectivity of the OEIP is not selective to specific cationic species, enhancing the risk that cation transport through the CEM is dominated by H^+ at low pH, leading to potential pH-related side effects. This phenomenon results in the problem stated above since significant shifts in pH lead to an abundance of H^+ , which are delivered along with the neurotransmitter affecting the channel selectivity and it can also create side effects in implanted applications.

6.2 Paper II: A proton trapping ion pump for selective drug delivery

In this Paper, we overcame the pH challenges by developing hybrid OEIPs that improve the delivery rate and selectivity of neurotransmitters commonly used to treat epilepsy. This was achieved by the introduction of palladium (Pd) proton trapping electrodes patterned along the ion-selective membrane of the OEIP. During delivery, the Pd electrodes absorb the H^+ into their structure and prevent them from being delivered to the target electrolyte, improving the effective OEIP efficiency for the intended substance delivery.

This study represented the first development and use of OEIPs with Pd proton traps (Figure 6.1). We showed that this device allows for selective delivery of the drug of interest while preventing undesired pH changes originating from delivered H^+ in a target such as biological tissue. Another important achievement from this study, was the near-perfect control of the amount of

delivered drug since we observed a direct correlation between calculated charge and GABA concentration (one to one ratio between electrons and GABA molecules). These new devices can improve the treatment of conditions such as epilepsy and chronic pain.

Figure 6.1: Schematic illustration of the Pd-OEIP. A) Schematic showing the operating principle. The application of a potential difference between the source and Pd/target electrolyte delivers GABA and H^+ . B) Inset showing that Pd at negative potential absorbs the H^+ into its structure and blocks them from being transferred further to the target electrolyte.

Contribution to this Paper: Developing fabrication techniques, performing electrical and chemical characterization, analysis and evaluation of the results, and writing the manuscript.

6.2.1 Planar Device Challenges

The demonstration of OEIPs demanded photolithographic techniques on planar substrates to sequentially pattern active and passive areas. For implantation applications, planar OEIP designs meet some limitations in the scale and the rigidity as well as issues related to invasiveness (planar substrates/geometry and flexibility).

In addition, the CEM or AEM channel of these planar OEIPs is fabricated via thin-film processing and can be adjusted based on a specific ionic resistivity, resulting in a geometry-dependent ionic resistance value. Planar devices with narrow channels present a higher resistance in comparison with wider channels for large ion transportation given the same length/width ratio. The high resistance is related to the tendency of narrow channels to present less swelling as compared to wider ones, providing less water uptake inside the IEM than is necessary to promote high ionic conductivity. The channel swelling strongly depends on the channel geometry, the concentration of the fixed charges and the water in the channel and the encapsulation. Fiber capillary ion pumps present control channel swelling and the concentration of the water in the channel is narrowly controlled.

6.3 Paper III: Overcoming transport limitations in miniaturized electrophoretic delivery devices

OEIPs and other such iontronic technologies have seen significant development and application in the organic bioelectronics' community, with applications ranging from cell signalling in vitro to abrogating seizures in epilepsy models in vivo. There are however major limitations in these devices' planar form factor: it is difficult to miniaturize the "delivery tip" to the scale of single cells, and the electrophoretic transport has only recently been explored in sufficient detail to provide reliable performance.

In this Paper, we demonstrated how these issues can be overcome with capillary-based devices (i.e., 3D channels rather than 2D thin-film channels) and systematic study of substance transport for the given geometry. In order to miniaturize the technology, we developed OEIPs based on glass capillary fibers filled with a polyelectrolyte (CEM). Capillary OEIPs facilitate the transport of larger ions such as drugs and neurotransmitters as a result of the larger ion-transport cross-section compared to planar devices. The main advantage of this design compared to planar OEIPs is that the delivery tip can be easily implanted in proximity to targeted cells and tissues. Capillary fibers can be

considered as a starting point for other fiber based OEIP and “iontronic” technologies enabling favourable implantable device geometries.

Here we reported a systematic investigation of ion transport through OEIP capillary fibers and the observed irregularities from the typical linear current-voltage behaviour by performing experimental characterizations in combination with computational modelling. Concentration polarization at the OEIP inlet (interface with the source reservoir) was found to cause the observed irregularities. This polarization drives electric field enhanced water dissociation at the inlet of the membrane. The experimental and simulation studies formed a significant correlation between the limiting ion current, the electrolyte concentration, and the diffusion coefficient of the ions. These results indicated a problematic point for transportation of many biologically relevant ions, since the delivery of most of the biomolecules typically requires low concentration solutions with, typically, low diffusion coefficients. The introduction of an ion-selective cap manufactured at the inlet provided a solution to this limitation and restriction by providing a significant increase of the inlet surface area of the membrane and thus the limiting current of the capillary fiber OEIP. The observed reduction of the concentration polarization by adding the ion-selective cap enabled miniaturized OEIPs to efficiently transport biologically relevant ions at low concentrations and at relatively much higher rates. This design enables a broader variety of biomedically relevant applications and needs such as applications related to neurological disorders and diseases where a wide window of drug delivery frequencies and amplitudes is desired.

Contribution to this Paper: Developing fabrication techniques, performing electrical and chemical characterization, analysis and evaluation of the results, and writing the manuscript.

6.3.1 Low Dose Delivery Challenge

Concentration polarization is a problematic point for the delivery of many biologically relevant ions, such as drugs and neurotransmitters since they are required to be delivered at low concentration doses and often have a low diffusion coefficient. Irregularities in the recorded current and water splitting phenomenon are enhanced for low concentration, making their delivery difficult.

6.4 Paper IV: Modulating inflammation in monocytes using capillary fiber organic electronic ion pumps

In this Paper, we reported the development of free-standing capillary fiber based OEIPs and their use in modulating inflammation in primary human monocytes in vitro. This study represented the first use of these new capillary OEIPs in mammalian cells or tissue and highlights the utility of this technology in the specific inflammation model as well for general drug delivery purposes. We demonstrated capillary OEIPs with the delivery of anti-inflammatory salicylic acid (SA) to primary human monocytes. SA delivery is shown to significantly decrease the production of pro-inflammatory cytokines (such as TNF α or IL-6) and provide an effective highly localized anti-inflammatory effect.

These results have broader implications in the use of iontronic devices for the study and treatment of inflammatory diseases, as well as for the understanding of the anti-inflammatory effects of SA on specific pro-inflammatory stimulation (here, via LPS). These results also pave the way for future applications of capillary OEIPs in other mammalian and human applications. The project was a collaboration with Robert Blomgran at the Linköping University's Department of Clinical and Experimental Medicine, where the in vitro study was performed.

Contribution to this Paper: Developing fabrication techniques, performing electrical and chemical characterization, analysis and evaluation of the results, and writing the manuscript.

7

Conclusions and Outlook

This thesis provides a brief description of organic bioelectronic technology based on the selective electrophoresis of charged ions through a polymeric channel. I focused on developing the organic bioelectronic technology and elucidating the ion transport process involved in these organic electronic ion pumps (OEIPs). Two designs of OEIPs based on polycation and polyanion materials were developed. Firstly, planar devices with the polyanion membrane PSS-co-MA were used to deliver small ions and more complex molecules such as the neurotransmitter GABA. Secondly, a novel design of OEIPs based on glass capillary fibers was developed to overcome the limitations of planar devices such as the delivery of large molecules with low mobility for implantable applications. Various form factors and functionalities were studied which were further used to optimize the ion transport efficiency of OEIPs.

7.1 Limitation and Challenges of OEIP Technology

In Paper I, we investigated the optimization of transporting the neurotransmitter GABA through OEIPs by adjusting the pH in the source electrolyte. We indicated that ion transport depends on the size, the mobility, and the configuration of the delivered molecule. GABA transportation is strongly dependent on pH conformation changes since the molecule passes from an extended structure to a tighter “folded” structure with the lowest free energy at pH 3. This knowledge of the consequences of pH shifting in the source electrolyte allows a broader variety of “pumpable” signalling or neuromodulator substances and, as a result, wider application of OEIP technology in basic research and future therapeutics.

A challenge in this study was the abundance of H^+ , which are transported along with the intended neurotransmitter due to the non-specific permselectivity of the OEIP, and potentially affects the biological medium. In Paper II, we developed the first OEIP based on palladium (Pd) proton trap electrodes to optimize the delivery of GABA neurotransmitters while reducing the proton transport. These results show that the new OEIP design integrated with Pd contacts can perfectly control the amount of delivered GABA and improve the effective permselectivity of the device since it allows delivery of specific ions while preventing undesired pH changes from protons delivery to the biological medium.

7.2 Development from Planar to Capillary Fiber-Based OEIPs

Planar OEIPs can successfully transport small ions with high spatiotemporal resolution. A great challenge during my PhD was finding a solution to deliver more complex and larger molecules that present a relatively high resistance for narrow or thin channels (planar OEIPs).

The delivery of these molecules requires a new device design with larger cross-sections to allow the selective migration of molecules through its solid bulk. Additionally, planar OEIP designs face some scale and rigidity limitations for implantation scenarios related to their penetration in a biological medium such as tissues or cells.

This thesis presents the first demonstration of capillary fibers-based devices (3D channels), which enable the delivery of larger molecules (drugs and neurotransmitters) deep inside tissues and cells. We developed miniaturized OEIPs based on glass capillary fibers filled with a polyelectrolyte (CEM or AEM) to deliver small ions, neurotransmitters (ACh), and drugs (SA).

7.2.1 Limitation and Challenges of Capillary Fiber OEIPs

It was a great opportunity to turn the challenge of delivering large molecules with low mobility at low concentrations into a great accomplishment. In Paper III, ion transport through capillary fibers was deeply investigated. The results show that the effect of concentration polarization at the inlet is more pronounced for relatively low electrolyte concentrations. The ion transport efficiency, in this case, is lower since transport is dominated by H^+ or OH^- produced by water splitting. This is an undesirable effect for most delivery devices and biological applications.

To resolve this issue, the inlet geometry was re-engineered by adding an ion-selective cap at the fiber inlet to increase the area of the electrolyte-CEM interface. This design allows the transport of large biologically relevant ions at low concentrations at relatively much higher rates and thus addresses a wider range of biomedically relevant applications and needs.

Paper IV shows the broader implications in the use of capillary fiber OEIPs for the study and treatment of inflammatory diseases. The anti-inflammatory effect of SA on pro-inflammatory stimulation was investigated in mammalian cells. The results of this paper show that highly localized SA delivery results in a significant decrease in cytokine levels with a low dosage, preventing the activation of inflammatory monocytes.

These findings clearly illustrate a beneficial approach to optimize ions transportation as well as local and specific delivery of different molecules and therapeutic substances, at low controlled doses for implantable OEIP applications with limited side effects.

7.3 Future Outlook

For the future of this work, it would be of great academic and medical interest to further exploit the possibility of local drug delivery to tissues. Planar OEIP studies must be explored more, in order to improve the devices' lifetime and the device operation for studies over extended periods of time.

Flexible substrates and parylene encapsulation can broaden the application range and facilitate the integration of devices to stretchable electronics for better interfacing with biological systems.

Additionally, capillary fibers can penetrate tissues and organs and they can be used for electrophysiology studies. A future goal is to use the next generation capillary fiber OEIPs coupled to cytokine transistor-based sensors to monitor the inflammatory process and deliver targeted doses of drugs using a feedback protocol to control delivery and activate the pump.

With this work, I dream to inspire a more rigorous investigation of the interface between biology and electronics, so as to fabricate more efficient bioelectronic devices that will be used in healthcare and tackle everyday health issues.

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