Conjugated Polymers for Neural Interfaces: Prospects, Possibilities and Future Challenges

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Doctoral Thesis
Trita-STH Report 2009:1

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Cover: Knitted neuron demonstrates the versatility of textile manufacturing techniques for the construction of three dimensional structures. © Maria Asplund

Academic dissertation which with permission from Kungliga Tekniska Högskolan (Royal Institute of Technology) in Stockholm is presented for public review for passing the doctoral examination on Friday January 30 2009, at 13:00. In lecture hall 3-221, Alfred Nobels Allé 10, Huddinge, Sweden

TRITA-STH Report 2009:1
ISSN 1653-3836
ISRN ISRN KTH /STH/ --2009:1—SE
Far better an approximate answer to the right question, which is often vague, than an exact answer to the wrong question, which can always be made precise." J. W. Tukey, 1962
ABSTRACT

Within the field of neuroprosthetics the possibility to use implanted electrodes for communication with the nervous system is explored. Much effort is put into the material aspects of the electrode implant to increase charge injection capacity, suppress foreign body response and build micro sized electrode arrays allowing close contact with neurons. Conducting polymers, in particular poly(3,4-ethylenedioxythiophene) (PEDOT), have been suggested as materials highly interesting for such neural communication electrodes. The possibility to tailor the material both mechanically and biochemically to suit specific applications, is a substantial benefit with polymers when compared to metals. PEDOT also have hybrid charge transfer properties, including both electronic and ionic conduction, which allow for highly efficient charge injection.

Part of this thesis describes a method of tailoring PEDOT through exchanging the counter ion used in electropolymerisation process. Commonly used surfactants can thereby be excluded and instead, different biomolecules can be incorporated into the polymer. The electrochemical characteristics of the polymer film depend on the ion. PEDOT electropolymerised with heparin was here determined to have the most advantageous properties. In vitro methods were applied to confirm non-cytotoxicity of the formed PEDOT:biomolecular composites. In addition, biocompatibility was affirmed for PEDOT:heparin by evaluation of inflammatory response and neuron density when implanted in rodent cortex.

One advantage with PEDOT often stated, is its high stability compared to other conducting polymers. A battery of tests simulating the biological environment was therefore applied to investigate this stability, and especially the influence of the incorporated heparin. These tests showed that there was a decline in the electroactivity of PEDOT over time. This also applied in phosphate buffered saline at body temperature and in the absence of other stressors. The time course of degradation also differed depending on whether the counter ion was the surfactant polystyrene sulphonate or heparin, with a slightly better stability for the former.

One possibility with PEDOT, often overlooked for biological applications, is the use of its semi conducting properties in order to include logic functions in the implant. This thesis presents the concept of using PEDOT electrochemical transistors to construct textile electrode arrays with in-built multiplexing. Using the electrolyte mediated interaction between adjacent PEDOT coated fibres to switch the polymer coat between conducting and non conducting states, then transistor function can be included in the conducting textile. Analogue circuit simulations based on experimentally found transistor characteristics proved the feasibility of these textile arrays. Developments of better polymer coatings, electrolytes and encapsulation techniques for this technology, were also identified to be essential steps in order to make these devices truly useful.

In summary, this work shows the potential of PEDOT to improve neural interfaces in several ways. Some weaknesses of the polymer and the polymer electronics are presented and this, together with the epidemiological data, should point in the direction for future studies within this field.
Dissertation

Paper I
Composite biomolecule/PEDOT materials for neural electrodes
Maria Asplund, Hans von Holst and Olle Inganäs
Biointerphases, 3,(3), pp. 83-93

Paper II
Biocompatibility of PEDOT/biomolecular composites intended for neural communication electrodes
Maria Asplund, Elin Thaning, Johan Lundberg, Ann-Christin Sandberg-Nordqvist, Beata Kostyszyn, Olle Inganäs and Hans von Holst
Submitted

Paper III
Stability of PEDOT materials intended for implants
Elin Thaning, Maria Asplund, Tobias Nyberg, Olle Inganäs and Hans von Holst
Manuscript

Paper IV
Wire electronics and woven logic, as a potential technology for neuroelectronic implants
Maria Asplund, Mahiar Hamedi, Robert Forchheimer, Olle Inganäs and Hans von Holst
Manuscript

Paper V
Incidence of traumatic peripheral nerve injuries and amputations in Sweden between 1998 and 2006
Maria Asplund, Mats Nilsson, Anders Jacobsson and Hans von Holst
Accepted for publication in Neuroepidemiology, November 2008

Related Work Not Included

Electronic circuitry integrated in fabrics
Mahiar Hamedi, Robert Forchheimer, Maria Asplund and Hans von Holst
DIVISION OF WORK BETWEEN AUTHORS

PAPER I
Composite biomolecule/PEDOT materials for neural electrodes
Asplund planned and performed all of the experiments and wrote the article under the supervision of von Holst and Inganäs.
Biointerphases, 3,(3), pp. 83-93

PAPER II
Biocompatibility of PEDOT/biomolecular composites intended for neural communication electrodes
Asplund planned the cell culture experiments together with Thaning and Kostyszyn. Asplund performed all of the polymer preparations and Asplund and Thaning both performed the cell culture experiments. Asplund was responsible for planning the animal experiments together with Lundberg, Sandberg-Nordqvist and von Holst. Asplund took part in animal surgery and performed immunohistochemical analysis together with Lundberg. Asplund wrote the manuscript.

PAPER III
Stability of PEDOT materials intended for implants
Asplund and Thaning took equal part in planning and performing the experiments under the supervision of Inganäs, Nyberg and von Holst. Asplund and Thaning took equal part in writing the manuscript.

PAPER IV
Wire electronics and woven logic, as a potential technology for neuroelectronic implants
Asplund wrote the manuscript, and performed the majority of experiments in collaboration with Hamedi and under the supervision of Inganäs, Forchheimer and von Holst. Forchheimer performed SPICE simulations.

PAPER V
Incidence of traumatic peripheral nerve injuries and amputations in Sweden between 1998 and 2006
Asplund, Nilsson, Jacobsson and von Holst planned the study. Asplund performed the analysis and wrote the manuscript under the supervision of von Holst.
PREFACE AND ACKNOWLEDGEMENTS

First of all I would like to thank my supervisor Hans von Holst for his never failing enthusiasm for this project, and for believing in me and my ability to do research. I would also like to express my deepest gratitude to my co-supervisor Olle Inganäs for inviting me to do research in the Biomolecular and Organic Electronics (BiOrgEl) group and introducing me to the field of conducting polymers. Special thanks to Robert Forchheimer for always taking time to answer my questions, teach me about electronic circuit design and for the crash course to the SPICE program.

Olle, Hans and Robert, it has been great to work with you. Thank you for letting me take part in your creativity and your scientific brilliance! I hope I will have the good fortune to continue doing so in the future.

I would also like to thank the following;

All the people in the BioOrgEl lab for making me feel like I truly belonged in your group.

All the people at the Section of Neurosurgery, Institution of Laboratory Medicine and Clinical Neuroscience, Karolinska Institutet, for you cheerful atmosphere. Especially, I would like to thank Britt Meijer, Johan Lundberg and Ann-Christin Sandberg-Nordqvist, for lots of hands on instructions with the in vivo experiments and immunohistochemical analysis.

Mats Ulfvendahl and Beata Kostyszyn at the Centre for Hearing, Karolinska Institutet, for lending us your lab space for cell culture during several years. Special thanks to you Beata for teaching me about cell culturing.

All my colleagues in the Neuronic Engineering group and the Imaging group at School of Technology and Health. Your support and encouragement throughout these five years have been exquisite and truly made this thesis possible. Thank you for making every working day so very pleasant!

Tobias Nyberg, for your excellent thesis that introduced me to this field. Thank you for joining our group and for always taking time to carefully investigate all my questions!

TACKORD

Jag skulle också särskilt vilka tacka följande personer:
Elin Thaning för din uttröttliga energi i allt du gör och för att du ändå alltid tar dig tid till en kaffepaus när jag behöver det.

Mats Nilsson, för ditt fantastiska tålamod i allt, och för ditt stöd genom åren.

Sofia Hedenstierna och Johnson Ho för att ni alltid tar er tid för en pratstund, och även nu de sista månaderna inte bara själva fixat era framläggningar med glans, utan även tagit er tid att peppa mig när jag behövt det.


Tack också alla ni utöver Olle och Hans, som hjälpt mig korrekturläsa; Axel, Tobias, Gary, Elin, Sofia, Peta, Andrej och Rickard, ni har alla gjort stora insatser för min sinnesfrid inför framläggningen.

Jag vill också tacka alla mina älskade vänner som alltid får mig på gott humör och för er bergfasta vänskap i alla lägen. Varmt tack till er alla! Elisabet, ett särskilt tack till dig som uppmuntrade mig att flytta till Stockholm och att söka mig till neuroprotesfältet.

Slutligen vill jag tacka min familj som alltid tror på min förmåga och stöttar mig när det behövs. Mamma, pappa, Anna, Kristina, Jens och Niclas, som alltid rycker ut med vad än jag behöver från sängplats till te. Andrej, särskilt tack för din fantastiska insats de sista månaderna då jag bara fick koncentrera mig på att skriva. Utan dig hade det inte gått!

Luleå, annandagen,
2008
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1 INTRODUCTION

The human body is controlled by a complicated signalling system, partly based on biochemical communication but also to large extent purely electrical signals. These signals are sent from the central nervous system through the peripheral nerves targeting everything from motoric muscular activity to our digestion. Electrical signals are also sent back from the body to the central nervous system giving feedback and information in the form of sensations, visual or auditory impressions. In this respect, electrical signals are fundamental for the function of our bodies as well as our experience of the outside world.

Anyone who has ever tried an electrical exerciser, or had an electric shock, knows that it is possible to stimulate the nervous system externally resulting in muscular contractions or sensations of pain. Through injection of electrical pulses in nerve tissue, it is possible to stimulate the nervous system artificially to elicit or control different body functions. Such neural electrodes are already used extensively in, for example, urinary bladder implants and deep brain stimulation and will, with further development, be extended to new areas of medical applications. One of the most well known neuroprosthetic systems in clinical use is the cochlear implant. Through patterned electrical stimulation in the inner ear, the cochlear implant has, for decades, provided otherwise deaf patients with hearing.

The use of neural communication electrodes is not interesting solely for the stimulation of nerves but also for the recording of neural activity. Arrays of recording electrodes, providing detailed access to neural signals, could be used to control external devices either from the peripheral nervous system or as a brain-computer interface. After a traumatic amputation, remaining parts of the nerve are still available for receiving and transmitting signals to the lost limb (Dhillon et al 2004) and substantial effort has been put into the development of electrodes capable of bi-directional communication with residual nerve tissue in amputation stumps. Future electrode systems capable of both recording and injecting neural signals could be used to implement prosthesis with actual brain controlled machinery, as well as artificial sensory feedback.

Stimulation of peripheral nerves (Guiraud et al 2006) or spinal cord (Mushahwar et al, 2007) in paraplegic patients can also be used for the restoration of movement in limbs. This might be a valuable tool for restoration of locomotion in otherwise paralysed patients in the future. It has also been shown that stimulation more centrally in the nervous system has the potential to create artificial sensations, like sight or hearing, in subjects otherwise completely blind or deaf. Electrodes stimulating either the retina (Humayun et al, 1999), the optic nerve (Veraart et al, 1998) or even directly in the visual cortex (Schmidt et al, 1996) have been shown to give visual impressions such as localised spots of light in profoundly blind subjects. Such studies bring hope that a parallel to the cochlear implant, for the retina, could be used to provide visual perception to blind patients.

In short, a bidirectional electronic interface between the human nervous system and external instrumentation, would not only provide a unique tool for the study of neural signals but also open up intriguing possibilities for a broad spectrum of applications, from mind-controlled prostheses to artificial sight or hearing. These kind of devices are often referred to as
neuroprosthetics, a term which includes applications targeting both the central and the peripheral nervous system. Neuroprosthetics are in general considered for the three main areas: restoration of motoric function; reconstruction of body reflexes; and artificial sensors. Even though several neuroprosthetic systems are already used in clinical practice, the full potential of such devices is not yet fully explored.

Although conceptually demonstrated in acute experiments by several authors, the main challenge for neural interfaces still remains. The route to success for numerous applications is the development of electrodes that have the electrochemical, biological and mechanical properties needed to safely and selectively communicate with the nervous system. Although requirements vary with application, the need for miniaturised electrode arrays, capable of interfacing with neurons over prolonged periods of time, is the common denominator and key needed to enable more advanced neuroprosthetic systems in the future. Until recently, the majority of work performed in the field of neuroprosthetic electrode arrays has been focused on traditional metals for electrodes and silicon microfabricated substrate structures. Extensive effort has been put into overcoming the obstacles of reversible charge delivery from miniaturised electrodes and the mitigation of implant glial encapsulation. A conclusion from much of this work is that, although partly very successful, the response of the immune system to these implants is a significant obstacle, complicating long term communication with neurons.

Recently the use of conducting polymers as neuroelectronic interfaces has become a topic of considerable interest. Rapid development in the area of organic electronics has opened up the possibility for a whole new set of electrode materials, the properties of which can be tuned at synthesis and hence tailored to suit specific applications. Originally, the conducting polymer polypyrrole (PPy) gained a lot of interest because of its high conductivity and reported biocompatibility. However, the poor stability of PPy electrodes has over time led to a change of focus to the more stable and highly conducting poly(3,4-ethylene dioxythiophene) (PEDOT), which previously has been extensively investigated for a diverse set of applications such as solar cells, printed electronics and light emitting diodes.

The primary aim of this thesis is to investigate the possibilities and possible limitations of PEDOT as a material for neuroelectronic interfaces. Although some fundamental questions of neuroprosthetic applications remain to be answered, it is clear that the introduction of conducting polymers offers new solutions, as well as new challenges, within the neuroprosthetic field.

Specifically, this thesis aims to tailor PEDOT for the neural interface through the inclusion of biomolecules in the polymer material. Experiments regarding biocompatibility, stability and electrochemical properties of the tailored material will address questions on how well this material matches the required properties of the neural electrode. Furthermore, the possibility to use the semi-conducting properties of PEDOT to include logic functions in the implant will be investigated. An epidemiological study will also lay the foundation of future work within specific applications.

It is essential that highly selective bidirectional neural interfaces are developed with an understanding of all the factors contributing towards successful clinical applications.
Therefore this report also attempts to give a brief but broad review of the fundamental questions involved in nervous system interfaces, although not all of them are directly linked to the introduction of conducting polymers.
2 THE NERVOUS SYSTEM

The nervous system is usually divided into two major groups, the central nervous system (CNS), constituting the brain and spinal cord, and the peripheral nervous system (PNS) constituting all the nerves in the body. Although the nervous system is built from many cell types, the nerve cells, called neurons, can be considered to be the fundamental building block. Neurons are specialised to receive, process and transmit signals to other cells and together with the endocrine system the neurons in the nervous system are responsible for controlling and coordinating all events of the human body. This ability is based on the fact that the neuron is an excitable cell.

The human body contains a wide selection of different excitable cells, both muscular and neural. Their common denominator is the ability of the cellular membrane to momentarily alter its permeability to ionic species and through this elicit sudden electrical activity over the membrane. The nervous system contains a variety of neurons and although their function and appearance differs, there are some general properties that unite them. In brief, a neuron has three main types of components; the cell body or soma, which is the central part of the neuron, the dendrites, which are mainly responsible for receiving input to the cell, and the axon, where signals are transmitted to other cells in the system (Figure 1a). When input to the cell raises the membrane potential over a certain threshold value, the neuron gets activated and communicates this as a signal to other cells. Along the axon signals are transmitted as an electrical impulse which, at the axon terminal, is chemically transmitted to the target tissue.

2.1 Ion channels, electrical excitation and action potential

The neuron membrane is equipped with both ion selective channels and ion pumps. When the neuron is in its resting state, diffusion, migration and active transport over the membrane build up ionic concentration differences between the cell interior and the extracellular fluid. This concentration difference also leads to an electrochemical potential difference over the membrane, called the resting potential. Particularly important ions withholding this potential are sodium and potassium ions, and at rest there is an excess of potassium inside the cell and an excess of sodium outside the cell. Typically, the neuron resting potential is around ~70 mV.

The ion selective channels in the membrane have dynamic properties related to the membrane potential. A sudden change in membrane potential affects membrane permeability through the opening of these channels. As a result ions flow across the membrane barrier; sodium ions flow into the cell balanced by an outward current of potassium ions. The opening does not necessarily affect all ion channels in the membrane. If the change is gradual, below the stimulation threshold, the fluctuation does not create any dramatical changes and the resting potential is shortly regained. However, if a stimulus elevates membrane potential above the threshold, a series of events take place. The strong ionic flow forces even more ion channels to open. The faster opening of sodium channels creates an initially stronger sodium flow into the cell, than the outward flow of potassium, raising the membrane potential to the positive side. Within a millisecond, the outward potassium flow
balances the sodium flow and, when further increased, membrane potential drops below the original potential. After closing of the ion channels, diffusion, migration and active transport once again build up the resting potential. If monitored by a hypothetical electrode measuring potential between cell interior and extracellular fluid, the course of events would give raise to the wave shape potential fluctuation shown in Figure 2. In general the process has peak-to-peak amplitude of around 120 mV, and is over in less than milliseconds, which explains the common expression that the neuron “spikes”.

![Diagram](image)

Figure 1: a) Sketch of nerve cell with a myelinated axon, b) signal transduction in a myelinated axon. Axonal membrane is only accessible for ionic passage at the nodes which lead to saltatory conduction. c) Cross section of a nerve enclosed by the epineurium and with subdivisions of axons organised into bundles by the perineurium.

2.1.1 Axonal signal conduction
The axonal conduction of impulses is not an electrical conduction in the sense of electronic conduction as in metals. Instead, the signal is transmitted as a series of excitation events taking place at the axonal membrane. Typically, an action potential is initiated as explained above, at the axon hillock, and consequently there is a sudden local ionic flow over the axonal membrane. This in turn gives rise to an electrical field spreading orthogonally to the ionic flow, resulting in electrical signal transmission along the axon (Figure 1b). The electrical impulse created at this point will spread along the axon, stimulating adjacent parts of the membrane on its way. Hence, the signal is longitudinally spread and at the same time amplified when new parts of the membrane open up to ionic flow along its path.
Figure 2: Action potential over nerve cell membrane. Peak-to-peak amplitude is around 120 mV.

Figure 3: The nervous system of the human body. 31 nerve pairs run symmetrically from the spinal cord, innervating every part of the body.
2.1.2 Axons in nerves

While some axons are sub micrometer in length, axons in the peripheral nervous system can be up to a meter, and are the functional component of what we usually refer to as nerves. Most nerve cell bodies in the peripheral nervous system are embedded in the spinal cord as grey matter. From there axons are bundled together in the 31 nerve pairs (left and right side symmetrical) leaping out of the spinal cord as shown in Figure 3. Distal to the spine, the bundled axons are branched into a multitude of thinner nerves targeting every part of the body. At the muscular cells, individual axons split into a high number of neural endings interfacing subunits of the muscle (motoric units) with the nerve cell. Thus, a nerve is a bundle of axons in some ways quite similar to an electric cable. The nerve cable is enclosed by a protective sheath, the epineurium, and inside the nerve subsections of axons are bundled within fascicles of connective tissue, the perineural sheaths (Figure 1c). Each axon within a perineural bundle is also individually embedded in separate sheaths, endoneurium, keeping the axons well protected and isolated from each other.

Fast nerve signal transduction is essential for the performance of the nervous system especially over longer distances, as is the case with most axons in the peripheral nervous system. For signal transduction along a nerve, a series of the two events, membrane depolarisation and axonal field spread, must take place where the former clearly is the time limiting factor. The sheaths surrounding the axons are, apart from protection and structural support, also important for enabling such fast axonal signal transduction. Most mammalian axons are enclosed by a supporting and insulating layer called the myelin sheath. This sheath is built up from supporting cells, Schwann cells, which wraps around the axon as an insulating layer, keeping the axon electrically insulated from its surrounding environment. Although the full length of the axon is covered in this cellular sheath, small parts of the membrane are left uncovered in between neighbouring Schwann cells (Figure 1a and b). This creates a regular pattern of openings to the membrane, the Ranvier nodes, along the myelinated axon. It is only at these nodes where interaction over the membrane can take place. Thanks to the effective barrier of the myelin sheath the decay of the faster field transduction is minimised, thereby reducing the number of the slower membrane depolarisations needed to forward the signal. The simplified image of the pulse jumping from node to node has lead to the term saltatory conduction to describe this mechanism. The internodal distance varies with cellular function and has empirically been found to correlate well to the axonal diameter. Generally it can be said to lie in the range of 1 µm. All axons are however not myelinated but un-myelinated axons are also part of the human nervous system.

Most nerves consist of a very large number of axons, but the number varies with function and proximity to the spinal cord. As a figure of merit the rat sciatic nerve, a common target for neural prosthetic experiments, contains around 27000 axons, the human acoustic nerve is estimated to contain around 30000 axons and the optic nerve around 1.2 million.
3 RECORDING AND ARTIFICIAL EXCITATION OF NEURON SIGNALS

For neuroprosthetic applications it is of equal interest to record, study and interpret neural signals as to artificially, through external stimulation, elicit neural signals of physiological meaning. Ideally, a neural interface should be bidirectional, i.e. both record and stimulate. However, recording of neural signals is in general a very different problem compared to eliciting them. Therefore the technical requirements on the electrodes also differ. While stimulation is mainly a question of optimising the stimuli pulse and material so that excitation is achieved while side effects are minimised, for recording purposes the central question is to maximise signal-to-noise ratio and maintaining signal properties. Hence, the electrode specifications might differ, while creating close contact with the object (nerve cell or axon) is essential for both purposes.

3.1 Recording of neural signals

Microscale applications like neuron communication pose many challenges, not just geometrically but the signals to be studied are very small. Even if the potential fluctuation over the membrane during excitation is around 120 mV, in vivo measurements in practice are on signal amplitudes in the size range 50-500 µV, depending on experimental conditions like electrode configuration and proximity to an axonal node. An important part of recording is that the signal expressed from the nerve is ionic and has to be converted to an electronic signal to be measured and conditioned appropriately. This means that the electrochemical events at the electrode/tissue interface will be important for signal transduction. Because of the low signal levels, many types of noise otherwise not considered, must also be included in the measurement model.

Ideally, one measurement point would be placed inside the axon, and one electrode on the immediate outside, to measure the actual potential difference over the membrane. Assume that such a measurement can be performed. The signal measured would, except for slighter deviations in amplitude and time, look like the typical action potential sketched in Figure 2. The peak-to-peak value would be around 120 mV and the complete signal pulse would be over in less than a millisecond. These kind of intracellular experiments are naturally very difficult to carry out in practice, and therefore several electrodes placed as close as possible to the tissue of interest are used instead. This means that it is no longer the actual membrane potential that is measured, but the significantly lower external electrical effects of the action potential on the surroundings. This signal decreases quickly with distance from the node, so electrical properties of the extracellular medium and proximity of electrode to target are very important parameters, as well as screening of external noise for the final quality of the signal. Even though it is commonly said that most relevant information recorded from neurons is around 1000 Hz (action potential takes < 1 ms), the actual frequency interval does span from 10 to 10000 Hz.
3.2 Artificial excitation of the neural cell

3.2.1 Strength-duration curve, chronaxy and rheobase

Excitation of a neural cell takes place when stimuli raises over a certain threshold value as explained in Chapter 2. The excitation threshold is related to both time and current in such a way that longer pulses can achieve excitation at lower currents than short pulses. In neurophysiology it is common that an empirically determined strength-duration curve is used to determine if a pulse is strong enough to excite the cell. The strength-duration curve describes the threshold current, $I_{th}$, for which excitation will take place if the cell is stimulated with a square pulse, i.e. constant current for a certain pulse duration, $T$. An example of a strength-duration curve can be seen in Figure 4a. The parameters rheobase (current, $I_{rh}$) and chronaxy (time, $T_{ch}$), can be determined from the curve, and are parameters describing the cellular sensitivity towards stimulation. Rheobase is defined as the asymptotic value of $I_{th}$, meaning the threshold current for stimulation with a pulse of infinite duration, while chronaxy is the pulse duration needed to reach threshold with a current of twice the rheobase. It can be of great assistance, to fit the curve in Figure 4 with an analytical expression. One way to do this is by using a hyperbolic expression on the form shown in Eq. 3-1 below.

$$I_{th} = C_1 \left(1 + \frac{C_2}{T}\right)$$

Eq. 3-1

The constants C1 and C2 can easily be rewritten as in Eq 3-2, considering the definition of rheobase and chronaxy and the appearance of the strength-duration curve.

$$I_{rh} = \lim_{T \to \infty}(I_{th}) = \lim_{T \to \infty}\left(C_1 \left(1 + \frac{C_2}{T}\right)\right) \Rightarrow C_1 = I_{rh}$$

$$2I_{rh} = C_2 \left(1 + \frac{C_2}{2T_{ch}}\right) \Rightarrow C_2 = T_{ch} \left(\frac{2I_{rh}}{C_1} - 1\right) \Rightarrow I_{th} = I_{rh} \left(1 + \frac{T_{ch}}{T}\right)$$

Eq. 3-2

The expression in Eq. 3-2 is commonly referred to as the Weiss equation. From this equation it can easily be calculated if a pulse is sufficiently large to excite the neuron just by determining the rheobase and chronaxy. Although other functions can be used to fit data, the Weiss equation is generally considered to provide the best fit to most data (Neuroprosthetics in Theory and Practice, 2005, Mogyoros et al, 1996) and therefore we will limit us to this equation for the following discussion.

It is often convenient to study the charge dependence of pulse length, instead of the current strength dependence. The expression for the threshold current $I_{th}$, needed to excite the neuron, can easily be translated to a linear expression for the corresponding threshold charge $Q_{th}$ as in Eq. 3-3. Hence, shorter pulses are more charge efficient for stimulation than longer pulses.

$$Q_{th} = T \times I_{th} = I_{rh} (T + T_{ch})$$

Eq. 3-3
Figure 4: Example of a strength-duration curve of nerve tissue, a) show the relation between threshold current and pulse duration (squared pulses) and b) show the relation between threshold charge ($I_{th} \times T$) and pulse duration.

Rheobase is depending on cellular size as well as several experimental properties like electrode distance to target cell, distribution of electrodes, orientation of cell and on surrounding tissue. This means that the measured value is highly variable (several orders of magnitude) for the same type of tissue in different experimental settings. Chronaxy value do not to the same extent depend on surrounding properties but is, compared to rheobase, more stable and is therefore often used to classify tissue excitability (Neuroprosthetics in Theory and Practice, 2005). In physiological meaning it is a measure of the responsiveness of a target neuron, to temporal features of an electrical stimulus, and knowledge of the tissue chronaxy is important for choosing correct stimulation parameters. For functional electrical stimulation purposes stimulation with pulse width close to the chronaxy is a rule of thumb. Durations surpassing the chronaxy do not significantly contribute to evoked response and are, in other words, a waste of energy (Tehovnik et al, 2005). Approximate chronaxy values for different excitable tissues in the human body can be found in Table 1.

Table 1: Chronaxy for different types of excitable tissue in the human body (Chapter 1.1, Neuroprosthetics in Theory and Practice, 2005).

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Chronaxy / ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skeletal muscle</td>
<td>0.1-1</td>
</tr>
<tr>
<td>Cardiac muscle</td>
<td>1-3</td>
</tr>
<tr>
<td>Smooth muscle</td>
<td>100</td>
</tr>
<tr>
<td>Myelinated nerve fibre</td>
<td>0.1-0.3</td>
</tr>
</tbody>
</table>
3.2.2 Frequency dependency of the strength-duration relationship

Although current strength and pulse duration are the main figures determining the excitation threshold, frequency is also a parameter that has to be taken into account (Veraart et al, 1998). Even though single pulses can be used to evoke neural response as described above, it is common to use trains of such pulses in electrophysiological studies. For reasons that will be more thoroughly investigated in Chapter 5, stimuli is often chosen as to first inject a certain charge (as in a rectangular pulse of current for a certain pulse width) and then immediately, or after a certain delay \( t_{IPI} \), followed by a pulse of reversed polarity withdrawing the same amount of charge from the interface. These pulses are then followed by an intermediate interval of zero current; the intermediate pulse distance, \( t_{IPD} \). The two phases can be specified individually by the parameters \( I_1, I_2, T1, T2, t_{IPI}, \) and \( t_{IPD} \), as shown in Figure 5. It is however very common to choose \( I_1= I_2, T1=T2 \) and \( t_{IPI}=0 \), and then the pulse can be specified simply by pulse amplitude \( I \), frequency \( F \) and pulse duration \( T \) (Figure 6). A decrease in threshold for higher frequencies, similar to the strength-duration relationship, can be seen in the strength-frequency plane as illustrated in Figure 7.

![Figure 5: Rectangular biphasic stimulation pulse with variable parameters, as defined in Veraart et al (1998).](image)

![Figure 6: Simplified biphasic stimulation pulse.](image)

3.2.3 Cable models of the neuron membrane

Although very useful for predicting neural response to simple current steps, the strength-duration relations discussed above tells little about excitability of the nerve for more complex pulse patterns. Through modelling the membrane with electrical equivalent circuits, the axonal response can be expressed through a system of differential equations allowing theoretical calculations on required pulse properties to achieve cell excitation. Hodgkin and Huxley (1952) were pioneers in this area and since their work in the fifties their models have been extended and refined by many research groups. Although not further included in this report, such methods have often been used to determine thresholds (Altman and Plonsey, 1990, Rattay and Aberham, 1993) and are valuable for the theoretical evaluation of different stimulation patterns (Sahin and Tie, 2007).
Figure 7: Strength-duration curve extended into the frequency plane as shown by Veraart et al. Reprinted from Brain Research (1998), with permission from Elsevier.
4 PRACTICAL INTERFACES WITH THE NERVOUS SYSTEM

4.1 Introduction
Although some neuroprosthetic applications only require a few electrodes for sufficient stimulation or recording, the possibility to communicate selectively with neurons is fundamental for the vast majority of neuroprosthetic devices. Regardless of whether the interface is intended for use in the PNS or in the CNS, basic design considerations are remarkably similar. Both types of interfaces aim to establish selective communication with single or smaller fractions of neurons, and maintaining that connection over prolonged periods of time. There exist a large selection of suggested interfaces and the following section will introduce some of the most common. Although spinal cord stimulation should formally be considered a CNS application, for this thesis, CNS interfaces are only discussed as interfaces intended to communicate with cortical neurons.

4.2 Cortical interfaces in practice
Brain machine interfaces and deep brain stimulation have been shown to give remarkably good results utilising only a few recording or stimulating electrodes. However, high density electrode arrays will be essential for future more advanced neuroprosthetic applications. The intriguing possibility of artificial sight through direct cortical stimulation is completely dependent on the development of safe and stable neuronal interfaces using several hundreds of individually addressable electrodes (Cha et al 1992, Thompson et al 2003). It is also not likely that electrical stimulation from the brain surface will allow either safe or selective stimulation in the range needed for such an application to function. Thus, these high density electrode arrays will also need to penetrate brain tissue in order to place the electrodes as close to the target neurons as possible (Normann et al 1999, Tehovnik et al, 2005).

4.2.1 Planar arrays
The first penetrating CNS electrodes were simply insulated metal wires with an exposed electrode site at the tip, thin but stiff enough to be inserted into the nervous tissue. Although several such wires placed within the tissue could be used to form arrays, more sophisticated methods, both for placement of the electrodes and addressability of single electrodes in multi electrode arrays, were needed. Through the introduction of silicon micro fabrication into this area (Wise et al 1970, Wise and Angell 1975), it was possible to batch fabricate arrays with several individually addressable electrodes. An additional benefit was that on-chip circuitry could be included in the array as well. Ever since, silicon micro fabricated probes have truly been a cornerstone in neural interface technology.

Silicon microfabricated arrays have a long history at the University of Michigan (Anderson et al 1989, Najafi et al 1985). One of the most extensively used silicon fabricated electrode arrays is the shaft microprobes commonly referred to as the “University of Michigan Probe”. Planar photolithography microfabrication is used to create silicon shafts patterned with electrode sites along the shaft. To facilitate insertion into brain tissue and minimize mechanical damage, shafts are provided with a pointed front and are fabricated as thin as ~15 µm. The probe can
be one single shaft, or have several shafts in parallel (Figure 8), to increase the accessible brain volume.

Figure 8: Sketch of a multi shank planar array, like the University of Michigan probe. Photo-lithography is used to create planar silicon structures patterned with electrode sites along the shafts.

Figure 9: The Utah Intracortical Electrode Array, a needle bed with 100 tips in a 10×10 matrix. This implant was originally developed for interfacing the cat visual cortex. Reprinted from Vision Research, Normann et al, (1999), with permission from Elsevier.

4.2.2 Three dimensional arrays
Cortical neurons are typically organised into columnar functional units. Each column is divided into a layered structure where each layer has a different function in the processing of brain signals. Neurons with similar functions are therefore found in layers parallel to the surface rather than radially distributed, which has important implications for probe design. For example, a system intended for artificial vision through cortex stimulation should provide patterned stimulation of neurons at same depth from the cortical surface, i.e. in the same cortical layer. Planar arrays like the University of Michigan Probe, is on the contrary designed to stimulate at different depth in the cortical tissue. Arrays with several shafts (Figure 8) have the ability to place a few electrodes on the same depth, and in addition stacked versions of the University of Michigan Probe have been suggested to increase the electrode density in each layer (Hoogerwerf and Wise 1994, Bai et al, 2000). Nevertheless, the need for electrode arrays specially designed to stimulate adjacent neurons on the same depth from the cerebral surface, led to the development of another set of silicon fabricated electrode arrays. Silicon microfabrication of “needle bed” shaped probes (Figure 9) was first developed at the University of Utah, and “the Utah Electrode Array” (UEA) has since then been an important tool for many neuroprosthetic researchers (Campbell et al, 1991, Jones et al, 1992). Their aim was to target the cat visual cortex, and for this purpose it was estimated that 100 electrode needle tips, 1.5 mm tall, placed in a square matrix spaced by 400 μm would be sufficient. Electrode tips were covered with gold or platinum, and polyimide insulated gold wires were bounded ultrasonically to the backside of the array.
4.2.3 Future perspectives of cortical neural interfaces

There exist a multitude of variations on the probe configurations discussed above, both commercially available and fabricated at different research facilities. Silicon microfabrication is often employed because it provides excellent opportunities for microscale precision, batch fabrication and the inclusion of on-chip circuitry. Even though chemical inertness of silicon is reported, a major problem has been the mechanical stiffness of silicon making micro motion (see Chapter 6) between the soft brain tissue and implant a detrimental process. This is believed to be one of the reasons for the thick encapsulation surrounding these implants. Although clusters of cortical neurons have been recorded with the UEA in acute experiments, (Nordhausen et al, 1994) and also some long-term recording and stimulation has been demonstrated (Normann et al, 1999, Rousche and Normann, 1998), the formation of an insulating cellular sheath encapsulating the probe is frequently reported as a reason for neuronal contact failure. Several authors describe the formation of this continuous and insulating scar (Turner et al, 1999, Szarowski et al, 2003), and Rousche and Normann (1998) even report that the encapsulation tends to slowly push the array out of the tissue in long term experiments. The capsule formation is not merely a consequence of the initial trauma caused by the insertion of the probe but is actively maintained by the immune system (Chapter 6). Recently, it has also been reported that apart from the thick glial capsule, neurons adjacent to electrodes also die as a result of the inflammatory response (Biran et al, 2005).

To suppress glial encapsulation, the materials sought are flexible and soft materials with mechanical properties similar to the brain tissue. This is compromised by the fact that the penetrating electrodes must be strong enough to allow insertion into the cortex without breaking, while at the same time thin and sharp to minimise cortical tissue displacement on implantation. The combination of these properties is a hard nut to crack. One way to circumvent this problem is to coat the needles with a stabilising material which can be dissolved once implantation is complete, as described with poly-glycolic acid wire electrodes by Stice et al (2003).

Polyimide as substrate material for two dimensional probes has been discussed mainly because of its demonstrated biocompatibility, its mechanical flexibility, possibility to incorporate on-board circuitry and the relative ease of which bioactive species can be attached to the surface (Cheung et al, 2007, Meyer et al, 2001, Pickard et al 1979, Richardson et al, 1993, Rousche et al, 2001). Even if polyimide can not be etched into high aspect-ratio needle structures like the UEA, the possibility to use planar patterning techniques and then folding into a three dimensional device, as shown in Figure 10 (Rousche et al 2001, Takeuchi et al 2004), could be one route to implement three-dimensional features from polyimide substrates.

4.3 Peripheral nerve interfaces in practice

Axons in the PNS are extensively protected by sheaths of connective tissue (Section 2.1.2). To record or stimulate PNS neurons, the most effective way to establish single unit communication is through finding ways to place electrodes within this connective tissue.
Alternatively one can attempt to distinguish separate axons, or functional groups of axons, from the more general signals that can be obtained using electrodes on the nerve surface.

Stimulation from the surface can to some extent be performed selectively, since nerve axons with different functions also have different transduction speed and sensitivity to stimulation. This means that selected types of axons can be stimulated prior to others and is, for example, the reason why it is possible to stimulate muscle contractions externally without inducing pain at the same time. Further, patterned stimulation on the nerve surface can make use of the fact that axons within the nerve are organised into bundles. This topography is in general connected to axonal destination rather than to functional assignment. The cross section of the nerve therefore has a certain geographical distribution, which can be of use for neuroprosthetic control through stimulation of separate motoric units. So called “selective inactivation”, using subthreshold depolarising prepulses to inactivate fibres lying close to the electrode, can be applied. This has been suggested as a possible route to access even deeper nerve fascicles (Veraart et al, 1993) using only electrodes on the nerve surface. However, for truly selective communication with small populations of axons electrodes should preferably be placed either between, or even within, the fascicles of the nerve. Such solutions come at the cost of higher invasivity, with several electrodes penetrating delicate tissue at risk of inflicting serious injury to the nerve.

In section 4.3.1-4.3.4, various suggestions on how to build multi electrode arrays for the peripheral nervous system are discussed. The terminology used by Navarro et al (2005) will be employed to categorise different types of solutions.

4.3.1 Extraneural electrodes

Single electrodes can be directly sutured onto the surface of peripheral nerves. This method can be used to place electrodes on the nerve surface for selective activation or recording, if the target fascicle is near the surface of the nerve trunk. For more selective stimulation it is however a prerequisite that several electrodes can be placed steadily on the surface and, with an increasing number of stimulating sites, it is practical to apply arrays of electrodes to the surface rather than several single units.

The most intensely investigated way to do this (Navarro et al, 2005) is by using cuff electrodes. This term is conventionally used for cylindrical electrodes enclosing the epineurium. These electrodes can be considered both for recording and stimulation purposes, and have been in clinical use over several decades for a number of applications such as urinary bladder implants and correction of dropfoot (Mortimer et al, 1995). Cuffs are also tested in functional electrical stimulation for walking in paraplegic patients (Guiraud et al, 2006), and even artificial vision through optic nerve stimulation (Veraart et al, 1998) has been suggested. As the name implies, a cuff electrode is a cylindrical device placed around the perimeter of a peripheral nerve. On the inside of the cuff, two or more electrodes are patterned on an insulating substrate material. The insulating material does not only act as a carrier for electrodes but also provides an outer delimiter of applied stimulation, thereby minimising cross-talk to other structures. Selective stimulation, by the use of cuff electrodes, has e.g. been demonstrated in upper extremity human nerves on patients undergoing upper extremity nerve repair surgery (Polasek et al, 2005). Some fascicular selectivity has also been
demonstrated in long term experiments on the human optic nerve (Veraart et al, 1998) and with half-cuffs around the peroneal nerve (Guiraud et al 2006).

A strong argument for the cuff model is that these electrodes are considered relatively safe, provided that the diameter of the cuff is large and flexible enough not to cause compression injuries. To achieve close contact with surface without conflicting safety, it is common to make the cuff as a self-sizing spiral device, able to adjust its diameter without imposing detrimental pressure on the nerve (Naples et al, 1988, Polasek et al, 2005), (Figure 11). Another clear advantage of the cuff electrode is that it can relatively easily be replaced upon malfunctioning.

A variation on the cylindrical cuff electrode is the Flat Interface Nerve Electrode (FINE) (Tyler and Durand, 2002). Through applying a small force over time the FINE flattens the nerve from the cylindrical shape to more oval. Thereby the fascicles are more accessible from the surface, activation thresholds are reduced and selectivity increased. This method has been studied on the sciatic nerve of cats (Tyler and Durand, 2002, Leventhal et al, 2006), and has been reported successful in reshaping the nerve without inflicting significant physiological or histological changes. Furthermore, increased selectivity has also been shown with this design (Leventhal and Durand, 2004).

4.3.2 Interfascicular electrodes
Despite the above mentioned studies showing selectivity with different surface electrodes, the resolution of these devices will still be lower than can be expected with more invasive solutions. Placing electrodes between the fascicles in the nerve is a compromise between the low invasivity of the cuff and the highly invasive interfascicular electrodes. Such interfascicular electrodes are intended to enhance selectivity of stimulation and recording compared to the surface electrodes of a cuff. Tyler and Durand (1997) suggested an extension of the cuff to a hybrid system, employing extra-neural cuff arrays complemented with interfascicular electrodes. Such a hybrid system would be capable of recruiting both surface regions and deeper fascicles selectively. Their implementation of this approach can be seen in the Slowly Penetrating Interfascicular Nerve Electrode (SPINE) shown in Figure 12. Electrodes are placed both on the inner surface of the cuff and on four penetrating elements which can slowly be forced through the epineurium by sliding of the closure tube. Only the loosely epineural sheet will thereby be penetrated, and not the perineural fascicles, due the much tighter tissue of the sheaths covering the latter. The authors report that functionally different stimulation was achieved with the interfascicular electrodes that complemented surface stimulation. Interestingly, they also report that threshold charges for recruitment were not significantly different with the interfascicular electrodes compared to the surface electrodes. It should also be noted that their experiments were acute and no long term results with these electrodes have been reported subsequently.

4.3.3 Intrafascicular electrodes
To achieve more direct contact with single axonal groups than in the devices described above, electrodes could also be placed within the neural fascicles. Intrafascicular electrodes can record from and stimulate axons with some selectivity within single fascicles, and with high selectivity between fascicles (Yoshida and Horch, 1993). This is a common method to
study peripheral nerves in so called microneurography. The ultrafine needles used in microneurography measurements are limited to acute measurements and, for actual implants, it is more common to apply a wire like electrode longitudinally inside the fascicle. These Longitudinal Intrafascicular Electrodes (LIFEs) are inserted through the epineurium and placed inside the nerve (Yoshida and Horch, 1993, Nannini and Horch, 1991), and selective recording and stimulation of both afferent (Dhillon et al, 2004) and efferent nerve fibres (Nannini and Horch, 1991, Yoshida and Horch, 1993) has been demonstrated with such devices. Selective muscular recruitment using pulses in the nC range has been reported and sensory feedback from nerves in stumps of amputees has been achieved by stimulation in the range of 5 nC (Dhillon et al, 2004).

Electrodes can be single wire unipolar electrodes or bipolar electrodes with dual wires (Figure 13). Naturally, this can also be extended to several electrodes, limited only by the practical and medical concerns of placement of large amount of wires within the nerve and the limited space for external wiring to individual electrodes. Problems encountered with LIFEs have been the damage inflicted on nerves due to either micromotion of stiff wires implanted in soft and mobile tissue, or tethering forces from external wiring. Similar to the encapsulation seen in CNS applications (Chapter 6), a build-up of fibrotic tissue over time complicates long term use of LIFEs. Therefore, in recent years the polyLIFE, a softer implementation using metallised Kevlar-fibres, has gained a lot of interest (Lawrence et al, 2003). Long term evaluation (6 months), in rabbit sciatic nerve (Lawrence et al, 2002), showed that although a capsule was still present, the more flexible polyLIFE yielded a significant reduction of capsule thickness compared to Platinum-Iridium wires.

Although results with LIFE electrodes are encouraging, highly selective stimulation and recording would presumably need more channels than is practically possible to implement using single wires. Penetrating silicon structures, like the UEA, have been suggested as a possible route to implant an array of electrodes intrafascicularly rather than single channels. Acute experiments in feline sciatic nerve demonstrated that single units could be recorded in this manner, which proves that the electrodes were indeed placed intrafascicularly (Branner and Normann, 2000). Selective stimulation was also reported at thresholds in the range 1.8 nC/phase. Long term (4-6 month) selective stimulation has also been investigated in a subsequent study (Branner et al, 2004), which showed that threshold levels, that initially were in the 1-40 µA range (200 µs pulse width), increased in several cases up to 100 µA as encapsulation progressed. Recording could also not be maintained after the first month due to build up of such insulating connective tissue around the electrode.

4.3.4 Regenerative electrodes

The need for truly selective neuron communication has led to the development of regenerative electrode nerve interfaces, which in short allows regenerating nerves to integrate with an electrode chip during its regeneration process. A thin membrane patterned with holes, inserted in-between the proximal and distal stump of a severed nerve (Figure 14), forces the nerve axons, attracted by the distal stump, to regenerate through the membrane holes (Mannard et al, 1974). Metallised electrodes around the holes will thereby be able to connect to single or small groups of axons. Prerequisites are that the membrane is thin and flexible enough, and is sufficiently perforated with holes, to allow for nerve survival despite
the invasive membrane. Such a membrane electrode is commonly referred to as a sieve electrode.

There exist numerous suggestions on construction materials for the sieve approach. As with CNS electrode arrays, silicon sieves gained much interest over the years due to the confirmed inertness in biological environments and the high number of patterning techniques available (Wallman et al., 1999). Despite numerous efforts, long-term success with silicon sieves has been hindered by inherent problems of successfully integrating the stiff silicon membrane in soft nervous tissue. Polymide has emerged as an alternative material for sieve electrodes (Stieglitz et al., 1997), and such implants show increasingly promising results over the last decade. Regeneration has been proven in long term evaluations (up to twelve months), nerve signal recordings with the polyimide sieve has been demonstrated up to seven months (Ramachandran et al., 2006), and selective stimulation has also been demonstrated (Ceballos et al., 2002).

Although results are promising for the polyimide sieve electrodes, regeneration over the sieve barrier do affect the nerve and it should be mentioned that there is a trade off between hole size needed for optimal regeneration and hole size desired for high selectivity (Lago et al., 2005). Successful regeneration in studies has also yet only been performed on dissected and regenerated healthy nerves in animals. It remains to be proven whether this hold true, also for injured nerves, e.g. after a traumatic amputation.

4.3.5 Future perspectives on peripheral neural interfaces

It is clear that microfabricated electrode arrays, whether they are in planar configurations like with sieves, or as penetrating electrode arrays, are important for establishing selective bidirectional nerve interfaces also in the peripheral nervous system. Although recent progress within the field of sieve electrodes is encouraging, it is also important to recognise that many neuroprosthetic applications already greatly contributes from simpler systems like the cuff electrodes. While a cuff electrode can relatively easily be exchanged, a sieve electrode is incorporated in tissue and can not be removed.

It is equally important to consider the biomechanical properties of implants in the PNS as in the CNS. Target peripheral nerves are situated in highly mobile structures and micromotion between stiff implants and softer nervous tissue is an important factor contributing to failure of peripheral nerve electrode contacts. Devices made from materials mechanically more similar to tissue therefore stand better chance of maintained functionality in long term applications. Planar patterning of flexible substrates, which can be folded into three-dimensional structures, should be interesting also for peripheral nervous system electrode arrays. This might be a possible route to construct cuff electrodes, with penetrating features similar to the SPINE, from single sheets of polyimide.
Figure 10: Polyamide patterned photo-lithographically and then folded into a three dimensional structure. Sketch of concept presented by Rousche et al (2001).

Figure 11: Schematic of a spiral cuff electrode consisting of an insulating substrate, provided with patterned electrode pads.

Figure 12: Schematic of the Slowly Penetrating Interfascicular Electrode, SPINE, as presented by Tyler and Durand (1997).
Figure 13: Schematic of a bipolar LIFE as presented by Nannini and Horch (1991). One stimulating surface is placed directly outside the fascicle, while the other wire is inserted longitudinally within the fascicle.

Figure 14: Schematic of a regenerative sieve electrode.
5 ELECTROCHEMISTRY OF STIMULATION IN NEUROPROSTHETIC APPLICATIONS

From the introduction in Chapter 3 it is clear that, to excite neurons and create artificial signals in the nervous system, stimulation should be done with charge pulses that exceed a certain threshold value. Stimulation pulse parameters must however be chosen carefully to ensure that stimulation is safe, selective and sufficient for long-term durable and functional nerve communication. First, one needs to consider which threshold level that must be reached to ensure excitation of the target nerve. As explained in section Section 3.2 this is not an absolute number but will vary with conditions like pulse length, charge density, cell to electrode distance and also between different neural tissues. Second, the electrochemical events at the electrode tissue interface poses upper constraints on the charge that can be delivered without imposing irreversible chemical reactions possibly detrimental to both electrode and surrounding tissue. Irrespective of electrochemical mechanisms involved, stimulation must also be kept within reasonable safety boundaries to avoid any neural, or other, tissue damage due to repeated pulsing. As with excitational thresholds, the vulnerability of cells to electrical stimulation also varies between different tissue types. A very brief introduction to stimulation induced tissue damage is therefore also included at the end of this chapter.

Finally, for selective stimulation, a high number of individual electrodes placed within the tissue are needed, which imposes upper constraints on electrode size. Due to all these contributors, there is no absolute safe limit that can be relied upon, but required stimulation is highly depending on application. However, summarising some of the papers published on these subjects, a few general directives can be identified.

5.1 Electrochemical processes involved in electrical stimulation and their implications for safe stimulation

5.1.1 The electrode tissue interface

A stimulation electrode is a device where electronic current flow, in electronics and leads, is converted into ionic current flow in tissue. A major concern for stimulation electrodes is to ensure that the translation from electronic stimuli, to ionic movement, takes place without undesirable electrochemical procedures as a side effect. Understanding of the electrochemical mechanisms involved in the electrode/tissue system is therefore of great importance.

When a potential, e.g. a stimulation pulse, is applied at the tissue interface, several electrochemical mechanisms can contribute to the transduction of the electronic pulse, in the solid state, into ionic current in the tissue. A potential signal can induce both a capacitive response over the interface, leading to the movement of charges in the liquid phase, and onset redox reactions at the metal/tissue boundary leading to a passage of charge from the metal to the liquid. The latter is often referred to as a Faradic process and means chemical reactions at the electrode surface absorbing or releasing charge. In reality, both these
mechanisms occur in parallel, and the extents to which each of these mechanisms contributes to the signal transduction depends on signal characteristics as well as material and microstructure of the electrode surface.

Simplified, the electrode/tissue interface can be described as a metallic surface interacting with a liquid electrolyte. If a positive potential is applied across the interface, positive charges in the metal and negative charges in the liquid build up a double layer at the metal liquid boundary. This layer, the Helmholtz double layer, acts as an effective interfacial capacitance that can be charged and discharged by switching potentials. In addition to the Helmholtz layer ions, transported to the interface through migration in the electric field, will build up an ionic concentration gradient. The balance between electrical attraction and thermal agitation away from the surface will lead to a Boltzmann distribution of charge, in this case an excess of anions and a deficit of cations, in proximity to the surface. This diffusion layer, first introduced by Goüy and Chapman, is hence a mixed double layer consisting of ions of both charges. Both the Goüy-Chapman layer and the Helmholz double layer contribute to the capacitance of the interface. In higher ionic strengths the Helmholtz double layer dominates the interfacial capacitance, and the influence of the Goüy-Chapman layer can be neglected. However, in physiological saline the Goüy-Chapman layer is in the range 10-100 nm, to be compared with the Helmholtz double layer which is in the range of 0.1 nm, and both should therefore be included in the total double layer capacitance. An alternating potential will charge and discharge this capacitance leading to ionic flux and thereby ionic current in the liquid.

5.1.2 The water electrochemical window

A problem which was identified early with electrodes for stimulation was that if the charge imposed on the system was high enough, gas bubbles were formed on the electrode and accompanied by strong local pH change. The reaction described is water electrolysis giving rise to oxygen gas and hydrogen ions, according to the following reaction (Eq 5-1):

\[ 2H_2O \rightarrow O_2 + 4H^+ + 4e^- \quad \text{Eq. 5-1} \]

If the potential delivered to the electrode is sufficiently high, the potential energy delivered will not only lead to ionic movements in the electrolyte through capacitive effects but also be used in oxidation reactions at the solid/liquid boundary. The electrolysis of water described above, is harmful to tissue both through the gas evolution and through its acidity. If a sufficiently negative potential is applied, the reduction of water could also occur as described by the formula (Eq 5-2):

\[ 2H_2O + 2e^- \leftrightarrow H_2 + 2OH^- \quad \text{Eq. 5-2} \]

Also, this reaction generates gas (H\(_2\)) and strongly influences pH of solution. To prevent water electrolysis, it was therefore stated that stimulation should be performed well within certain voltage boundaries, ~0.6 V to 0.8 V vs Ag:AgCl, which was defined as “the water window”. The water window thereby places constraints on how much potential that can be used for current delivery to the tissue, before gas formation (either H\(_2\) or O\(_2\)) and pH alterations threatens to induce injury to surrounding tissue.
5.1.3 Reversible charge injection through biphasic stimulation

Naturally, electrolysis of water is not the only redox reaction which can take place during the electron-to-ion transfer at the neural interface. Any charge delivered to the interface will be a potential oxidiser and the potential range applied determines what reactions are thermodynamically possible. These reactions can be either corrosive reactions of the electrode material, or redox reactions in the media surrounding the electrode, i.e. biological tissue or electrolyte. Corrosive redox reactions in the long run lead to dissolution of the electrode material (Donaldson and Donaldson, 1986) and for in vivo applications it is not just a question of eventual device failure, but also electrochemical by-products may in fact be toxic to surrounding tissue. Redox reactions in tissue or in body fluids alter the chemical composition and can thereby create undesirable side effects of stimulation. Hence, the water window constitutes only an outer limitation on applied potential, and other redox reactions occurring within this window must also be taken into account.

The keyword for long-term stability, despite redox activity, is reversibility. The principle of safe charge injection through balanced biphasic pulsing was first presented by Lilly et al in 1955. Using symmetrical current waveforms, Lilly et al showed that stimulation induced tissue lesions could be prevented through applying symmetrical biphasic stimulation pulses instead of repeated pulsing with the same polarity. The idea is to stimulate through pulse pairs, so that every pulse is immediately followed by its reverse withdrawing the same amount of charge, from the interface, that was delivered by the first pulse. The second pulse will reverse chemical reactions inflicted by the first pulse and therefore ensure that the net electrochemical effect on electrode and tissue is zero. All redox reactions are however not reversible in practise. Total reversibility presumes that the reversed pulse is applied on the same species that was the result of the first pulse, which can not be taken for granted. In the case of water electrolysis the reaction is clearly not reversible in this aspect considering the evolved gas will not remain at the electrode. Also with reactions not involving gas evolution, molecules can migrate from the interface before reversion is allowed to take place. Thereby, even if mitigated, there will still be a net build up of electrochemical by-products in the vicinity of the electrode. Both the applied potential, the duration of the pulse and the material of the electrode determines if this net effect can be neglected. The choices of electrode material and pulse parameters thereby become of utmost importance to ensure safe stimulation with charge balanced biphasic pulsing.

If the product of the redox reaction is immobilized on the electrode surface, reactions approaching true reversibility can be established. Using noble metal electrodes like platinum or gold, two types of surface bound reversible redox reactions; surface oxide formation or H-atom plating, can contribute to such safe charge transfer (Brummer et al, 1975). Examples of such reactions for platinum are found below in Eq. 5.3 and 5.4:

$$Pt + H_2O \Leftrightarrow PtO + 2H^+ + 2e^-$$  \hspace{1cm} \text{Eq. 5-3}

$$Pt + H_2O + e^- \Leftrightarrow Pt - H + OH^- \Leftrightarrow Pt^+ + OH^- + e^-$$  \hspace{1cm} \text{Eq. 5-4}
The extents to which each of these processes can contribute to the current response also depend on the electrolyte, i.e. the extracellular tissue fluid. Other ionic species might participate in redox reactions within the water window and occur in parallel with, or interfere with, the oxidation or H-atom plating. The whole system, i.e. both electrode material and electrolyte, must therefore be taken into account when determining which pulse amplitude and duration that can be considered to be safe and reversible.

Continuing the example of platinum electrodes in extracellular tissue fluid, chloride ions can be expected to participate in corrosive reactions with the platinum surface. The corroding reaction in Eq. 5-5 is onset prior to oxygen evolution, and contributes to irreversible corrosion of the electrode (Donaldson and Donaldson, 1986).

\[
\text{Pt} + 4\text{Cl}^- \leftrightarrow \text{PtCl}_4^{2-} + 2e^- \quad \text{Eq. 5-5}
\]

For example, the initial estimation of maximum safe charge injection with platinum, given by Brummer and Turner (1977), was 350 µC/cm². Their measurements considered mainly the boundaries set by the water window. In a later study Donaldson and Donaldson (1986) showed that platinum dissolution in physiological saline could corrode to the extent of 20 µg/C, even when charge balanced biphasic stimulation well within the water window was used; a rate which would be catastrophic for a microsized electrode intended for long term use. For platinum in physiological saline an upper restriction of 0.6 V vs SCE was therefore suggested, which seriously limited the safe charge injection to only a tenth of the value originally suggested by Brummer and Turner. It should be noted that platinum dissolution has subsequently been shown to be inhibited by protein adsorption to the surface (Robblee et al, 1980) and present recommendations for safe charge delivery are in the range of 50-150 µC/cm² (Rose and Robblee, 1990, Neuroprosthetics in Theory and Practice, Chapter 3.1).

An attractive property of iridium oxide is its ability to transition through several oxidation states which can contribute to the reversible charge injection limit. Films of iridium oxide can be formed through repetitive cycling of iridium and a much more powerful reversible redox system, than the one described for platinum above, is thereby provided on the electrode surface. Iridium oxide film has therefore been an invaluable electrode material for neuroprosthetic applications over decades (Beebe and Rose, 1988, Robblee et al, 1983), and their reported maximum safe charge injection limits range from 2.0 to 4.1 mC/cm² depending on criteria selected as safe and on film deposition techniques (Beebe and Rose, 1988, Lu et al, 2008, Slavcheva et al 2004, Weiland et al, 2002). Maximum charge injection limits as high as 9.6 mC/cm², using potential biased asymmetric pulses, have also been reported (Cogan et al, 2006) for iridium oxide. It is clear that safe charge injection from iridium oxide films by far exceeds platinum. However, it should also be noted that not all studies take into account additional reactions apart from water electrolysis that could contribute to degradation, but assumes voltages within water window to be safe to apply. Additional degradation reactions in situ can therefore not be excluded, and irreversible changes at repeated pulsing in vivo has been reported at charge densities in the range 1.3 mC/cm² (Weiland and Anderson, 2000) to 3 mC/cm² (Cogan et al, 2004). Lu et al (2008) also report a 2 % decrease in electroactivity of iridium oxide films after only 12 h of stimulation at 4.1 mC/cm². Although they report this as a sign of film stability, it is clear that long term stability of iridium oxide films should be evaluated adequately, i.e. months to years both in...
*vitro* and *in vivo*, to ensure stability over relevant time frames at these high charge injection ratings.

In theory, charge balanced bi-phasic pulsing is an excellent way to deliver charge repeatedly, minimising detrimental side effects on electrode as well as surrounding tissue. Profound electrochemical evaluation of the tissue/electrode interface and the possible side reactions to injected charge must be undertaken, to ensure that irreversible mechanisms are neglectable in size. A recent study on current density distribution at the interface also suggests that merely keeping track of the charge balance is not sufficient for reversibility. Different current density distribution at the electrode, for different amplitudes in the cathodic and anodic phase, might disable the charge balance mechanisms and lead to a build up of electrochemical by-products despite charge balance and assumed reversible mechanisms (Cantrell *et al.*, 2008). This should be kept in mind when asymmetric pulses are considered, for example as when boosting the charge injection of iridium oxide.

### 5.1.4 Capacitive charge delivery through the Helmholz double layer

From the discussion in Section 5.1.3 the advantage of using only the double layer capacitive mechanism for charge delivery is evident. Double layer charging and discharging takes place without charge transfer over the interface and the process is completely reversible. The crucial question is if the pulse transmitted through this mechanism can be strong enough to elicit neural excitation. In other words, can the capacitance mechanism deliver sufficient charge for stimulation of neurons, with potentials low enough not to induce unwanted electrochemical side reactions? Charge delivery, through the capacitive double layer, can be expected to be in the range 20 µC/cm² (Neuroprosthetics in Theory and Practice, Chapter 3.1), and is in other words small compared to the charge delivery that can be achieved through redox reactions on the surface.

Since many applications desire charge deliveries markedly above the double layer capacitance, numerous efforts have been made to increase the capacitance of the double layer for different metal electrodes. Already in 1897, the German scientist Kohlrausch electrolytically deposited finely divided platinum to the surface of platinum electrodes to create a rough surface and thereby an increased surface area. If the interfacial area of the electrode is increased, the area of the Helmholz double layer is also increased and thereby capacitance. In 1963, Schwan managed to lower the electrode impedance of a platinum electrode about 30 times using a technique based on this principle. The resulting material, known as Platinum Black, has found extensive use both in electrochemical measurements and as material for pacemaker electrodes. Other suggestions on how to increase the effective interfacial area has followed. Recent advances show the potential of carbon nanotubes as a possible route to increase interfacial capacitance (Keefer *et al.*, 2008) and numerous similar suggestions exist.

Another way to increase the possible capacitive charge delivery is to use very thin layers of insulating dielectric media effectively blocking the interface, thereby allowing no Faradaic charge transfer but only the capacitive mechanisms described in Section 5.1.1. For effective capacitive charge transfer it is essential to find materials that have the dielectric properties to, in thin layers (thickness in the µm range), efficiently block passage of Faradaic charge at the required potentials. The thinner the dielectric the higher the capacitance, but this comes at
the cost of a lower breakdown voltage. Since capacitance is directly proportional to area, it is also desirable that the electrochemically effective area is high. There exist numerous materials that can be used to implement such purely capacitive interfaces. A common example highly relevant for neuroprosthetic electrodes is Titanium Nitride (TiN). Although there have been reports of charge transfer in the range 22 mC/cm², Weiland et al (2002) suggests more conservative limits in the range 0.87 mC/cm² for TiN. It has been suggested that the high variability of safe limits could be due to differences in sputtering deposition and electrode size. It should also be mentioned that Weiland et al measured “safe” charge injection as the charge which can maximally be injected using charge delivery with potentials within the water window. However, the decision of which stimulation that can be considered safe might be more influenced by the potentials where the dielectric layer breaks down than to water electrolysis, since Faradaic charge transfer is blocked. It is clear that for TiN, as well as for Iridium Oxide, long term evaluation will ultimately determine what safe limits that should be applied for TiN electrodes.

5.2 Stimulation induced tissue damage

5.2.1 Evidence for stimulation induced tissue damage

It has been shown that stimulation of neural tissue can inflict injury to the cells, also with stimulation within the electrochemical safe limits. The manner and degree to which stimulation induced neural injury occurs, depend upon the physiological and pharmacological composition of the target neuron as well as the stimulus parameters. To further complicate the matter there are also difficulties to differentiate between electrochemically and activity induced tissue damage in most studies. Tissue damage due to the passage of current may arise from electrochemical by-products formed at the electrode-tissue interface and/or from physiologic changes in the tissues that are associated with the neural excitation itself (Grill 2005, Agnew et al, 1993). Evidence that actual non-electrochemically induced tissue damage exist comes from studies showing that blocking of action potentials from cells during electrical stimulation can protect the neuron from injury, which implies that the injury is related to the actual neural activity in itself (Agnew et al, 1990). In the following text, injuries related to electrochemical processes at the electrode/tissue interface will not be considered, but solely injuries which are directly related to the flow of current through tissue.

Despite numerous efforts to determine threshold levels for neural damage, in both histological and functional studies, consistent numbers have not been found. Levels that in one study have been found to induce no detectable changes by histological evaluation have, in other studies, been found to reduce the cellular response to stimulation in such a way that stimuli levels must constantly be raised to induce the same neurological response. Reasons for this uncertainty is the great variation in animal models, methods to analyse if injury occurred and the span of stimulation parameters applied in different studies. The methods of evaluating the outcome range from purely histological end point evaluations to behavioural studies observing the presence or lack of reflexes coupled to the nervous structure of interest. There exist few studies on human subjects and slight alterations in stimulation outcome are therefore difficult to detect. Earlier studies, employing only histological end-point evaluation of tissue damage, reported much higher thresholds for stimulation induced
injury than subsequent studies employing behavioural response to stimuli to evaluate change in excitational thresholds over time.

There are many parameters to take into account when predicting potential injuries of a certain stimulation pulse or pulse train. Charge density of a stimuli, as well as charge per phase in a stimulating pulse train, was in the 1990ies found to strongly correlate with tissue damage (McCreery et al., 1990, Shannon 1992). Shannon suggested a model based on the experimental data from McCreery, where injury threshold was determined from both these parameters through the following relation:

$$\log(D) = k - \log(Q)$$  \hspace{1cm} \text{Eq. 5-6,}

where \(D\) and \(Q\) represents charge density (\(\mu\text{C}/\text{cm}^2\)) and charge per phase (\(\mu\text{C}/\text{phase}\)) respectively, and \(k\) is a constant determined from McCreerys experimental data. Setting \(k\) to \(~1.5\) was found to give a conservative safe limit. However, this model also has two serious limitations. First, frequency of stimulation is not covered by the model and, as will be explained in the following section, subsequent experimental data has clearly shown the importance of frequency in this matter. Second, McCreery used only histological evaluation to detect injury which does not reveal more subtle changes like excitational threshold elevations. Stimulation-Induced Depression of Neuronal Excitability (SIDNE) is a well known effect of prolonged stimulation, also in the absence of histologically detectable injury (McCreery et al., 2000, McCreery et al., 1997). The charge densities suggested to be well within the safety margin by the Shannon model, ranges up to 30 \(\mu\text{C}/\text{cm}^2\) for the smallest values of charge per phase and might still produce SIDNE within hours of stimulation onset.

5.2.2 SIDNE and its implications on safe stimulation

After a period of continuous stimulation, the excitational threshold of the neuron is often raised, most of the time reversibly but some times also irreversibly elevated. The SIDNE phenomenon occurs even at stimulation with charges only slightly above excitational thresholds (McCreery et al., 1997) and applies only to neurons directly excited by the stimulation. SIDNE is believed to be related to mass action, meaning a mechanism where cells are injured when a sufficiently large number of axons are recruited simultaneously. Presumably this could be due to the sharp change in extracellular environment produced when many axons are activated at the same time (McCreery et al., 2002), or alterations in the vulnerable intracellular milieu.

Stimulus amplitude as well as temporal aspects of the stimulation like frequency, pulse duration and stimulus duty cycle, affect the occurrence of SIDNE (McCreery et al., 1997, McCreery et al., 2000, McCreery et al., 2002). Simply increasing the stimulation charge to compensate for SIDNE is not a viable approach, but has been found to worsen the condition and thus is both ineffective in the long run and increases risk of irreversible injury to neurons (McCreery et al., 2000).

Data published by McCreery et al (1997), from behavioural studies of stimulation in the cat cochlear nucleus, suggest that stimulation at high frequency is a strong contributor to SIDNE. Stimulation at 100 Hz, 100% duty cycle and 3 nC/phase (microelectrode, 500 \(\mu\text{m}^2\)),
did not lead to increased behavioural thresholds. However, a frequency raise to 250 Hz for the same charge showed clear occurrence of SIDNE after 7 h, despite the fact that duty cycle was lowered from 100% to 50% to compensate for the higher frequency. The same study also showed that decreasing the duty cycle attenuated threshold increase and therefore suggests that high frequency stimulation is rotated between several neighbouring electrodes, thereby reducing the duty cycle at each individual electrode but maintaining temporal resolution. Obviously, the downside of such an approach is the reduced spatial selectivity of stimulation. The optimal solution to this trade off, between high stimulation frequency on one hand and high selectivity of neuron populations on the other, is highly dependent on the application. A subsequent article from the same group (McCreery et al, 2002), studying excitability of cortical neurons in cats, report no changes in thresholds when pulsed with 4 nC/phase and 50 Hz for 7 hours at single electrodes. The same parameters applied simultaneously at all 16 electrodes did however significantly raise thresholds, which implies that simultaneous activation of many closely spaces electrodes do exacerbate SIDNE. This suggest there might be a limit to spatial selectivity of electrical stimulation also when rotated stimulation is not employed, or at least the importance of choosing pulse parameters that will not overlap stimulation fields of neighbouring electrodes.

Much of what is known about SIDNE resides from studies of the cochlear nucleus. From cochlear implant technology it has been determined that high frequency stimulation gives better sound experience for the patient. For many other neuroprosthetic applications human trials have only just been initiated. Whether the same high frequencies used for stimulation of the cochlear nucleus will also be employed for other neuroprosthetic applications in the future, remains to be determined. For example, Humayun et al (1999) report frequencies in the range 40-50 Hz to produce continuous, non-flickering sensations of light in stimulation of human retina. Different neural tissue can also be expected to have different tolerance levels for when SIDNE occurs. In summary, until more profound understanding of the SIDNE mechanisms enables reliable theoretical predictions of injury thresholds, experimental evaluations for each individual application should be performed and are especially important when high frequency stimulation is employed. When designing dense electrode arrays, it should also be taken into account that simultaneous activation of many closely spaced electrodes does increase the SIDNE effect.

5.3 Summary

It is beyond the scope of this thesis to present all electrode materials that have been suggested for neural electrodes. This chapter introduced the general concepts of safe charge delivery and present state of the art materials including their properties. Conducting polymers were not included in this chapter although they posses interesting properties that can be used for safe charge injection. This will instead be thoroughly discussed in Chapter 8. It is evident that the amount of charge per phase that is possible to inject safely from an electrode surface depends on which electrochemical reactions that are considered tolerable to participate in the charge transfer. Using the water window as only criterion for safety might not be sufficient, and the most trustworthy way to ensure electrochemically safe stimulation is through careful investigation of electrodes and tissue after actual stimulation, long term in vivo. One should also bear in mind that electrodes made from the same materials still differ in their actual safe charge injection, depending on parameters like microstructure and as is the
case with iridium oxide, the amount of activated oxide and the deposition method used. Furthermore, just staying within the electrochemical safe limits for stimulation is no guarantee for long term stable interfaces with neurons, and histological end point studies will not suffice as the only method of evaluation of neuronal injury. It is possible that long term safe stimulation will require a rotation of the stimulation pulse between several closely spaced electrodes to prevent accumulating threshold elevations.
Placing an implant in living tissue will always imply an initial tissue injury due to the surgical implantation; wound healing of this injury and subsequently the host reaction to the persistent implant, “the foreign body response”. The nature of the foreign body response depends on factors like implant size and shape, initial injury, material and surface properties, and of course site of implantation. The end-stage of this response is in general the formation of a fibrous capsule protecting the tissue from the implant and, as is the case with neural interfaces, unfortunately also efficiently insulating the electrodes. The foreign body response in the nervous system is therefore a significant hindrance for long term stable electrical neural interfaces. The following chapter will briefly describe what is known about the foreign body response to neural interfaces in the CNS and the current strategies investigated to mitigate this capsule formation. The PNS foreign body response is not further discussed here, but could presumably contribute from the same factors that can be used for mitigation of encapsulation in the CNS.

6.1 CNS response to implant

Since the introduction of silicon microelectrode array cortical implants, long term in vivo recording and stimulation experiments have become increasingly common. Although the inertness of the silicon material itself has been fairly well established in the biological environment, the insulating capsule formation has emerged as a persistent problem. This often leads to failure to record or stimulate efficiently after the first month and, in some cases, probe displacement in tissue over time. Great variability in success between identical implants in different animals and even between implants in the same animal, has led to an increasing need to fully understand the factors influencing this capsule formation and how it can be minimised.

The cortical response to an implant can be said to have two separate phases. First, insertion trauma including cutting through cells, extracellular matrix and possibly also vessels, sets of a wound healing reaction recruiting astrocytes and microglia to the site of the injury. Hemorrhage and edema follows the initial injury but in general diminishes within the first weeks. Macrophages arrive with the blood stream, while microglia migrates from surrounding tissue to the injury site and are activated into a macrophage like phenotype (Fawcett and Asher, 1999). This preliminary injury phase, assumed to be similar to that of a stab wound, is transitory (Polikov et al, 2005) and the affected tissue volume naturally depends on the size of the device and also to some extent the method of insertion (Szarowski et al, 2003). After the first phase, often called the “acute response”, follows a non-transient and more localised response to the foreign body. The persistent presence of the implant inhibits full healing and lead to encapsulation of the foreign object and eventually chronic inflammation.

In short, activated astrocytes in the vicinity (~500-600 µm) (Turner et al, 1999) of the implant migrates towards the injury site and organises into a mesh of hypertrophic astrocyte processes. Within the first weeks this region is loosely organised, and individual astrocyte
processes are clearly distinguishable. It has also been reported that during this first period, up to four weeks post insertion, the astrocytic scar formation is adherent to the implant surface (Turner et al, 1999). With time the affected region decreases while the scar becomes denser, and at six to twelve weeks individual processes are no longer distinguishable but are part of a tight non-adherent sheath effectively sealing the implant. This seal, or capsule, is often referred to as the glial scar and is suggested to form largely independent of the extent of initial insertion trauma (Szarowski et al, 2003). This time course also corresponds well with clinical observations of deep brain stimulation electrodes (Yousif et al, 2008). It has been suggested that meningeal fibroblasts migrating along the device is an important contributor to the glial scar (Fawcett and Asher, 1999) and that the presence of fibroblasts around the implant correlates with cases of more extensive scar formation (Seymour and Kipke, 2007, Kim et al, 2004).

Although glial scarring is an expected wound healing response after injury in the brain (Fawcett and Asher, 1999), the gliotic scar around an implant is different from what would be expected after a stab wound. Apart from astrocytes, microglia have also been shown to be a persistent part of the scar tissue. This would be expected in the initial wound healing response, but would not be found continuously in the scar after a stab wound (Szarowski et al, 2003, Biran et al, 2005, Biran et al, 2007). It is hypothesised that the presence of the foreign body leads to continued activation of microglia attempting to phagocytise the implant and to a chronic inflammatory reaction (Biran et al, 2005).

Reported capsule formations around active silicon implants are in the range 50-100 µm (Szarowski et al, 2003). Furthermore, from resistance measurements on capsules in the peripheral nervous system, the resistivity of encapsulation can be expected to be in the range 600 Ωcm (Grill and Mortimer, 1994). This implies that the capsule is a significant barrier that has to be overcome both for recording and stimulation. It has also been shown that, apart from the glial scar, neuron density in the area closest to the implants is significantly attenuated. Suggested mechanisms are 1) the initial trauma creates a “kill zone” of neurons close to the implant, 2) development of gliotic scar pushes neurons away from the implant or 3) tissue reaction creates an unfriendly or even toxic environment for neurons. Comparisons with stab wounds shows that the initial “kill zone” due to insertion trauma is much smaller than the zone of neuronal loss around the implant (Biran et al, 2005). Furthermore, high amount of ED1+ cells around the implant correlates well with large kill zones and much point to that the continued activation of microglia, if not the only contributing factor to this neuronal loss, at least is responsible for a large part (Hanish 2002, Biran et al, 2005). Neuronal loss from the vicinity of the electrode mean that, apart from the insulating barrier of the glial scar, the distance to closest neuron is also further increased often leading to failure to record, increased stimulation thresholds and decreased selectivity.

6.2 Summary and general guidelines for reduced glial scarring
As mentioned, the thickness of the glial scar and also the extent of microglial activation varies between experiments, even when experimental conditions are very similar. Some authors report scarring so intense that implants are eventually pushed out of tissue (Rousche and Normann, 1998) or driven downwards in tissue (Liu et al, 1999), while others report only a monolayer of glial cells surrounding the implant and healthy neurons in close proximity.
(Edell et al., 1992). Some general guidelines have resulted from work during the last decades. First, electrode arrays tethered to the skull induce more tethering forces between implant and brain leading to strain related injuries on surrounding tissue. Therefore, free floating electrode arrays, i.e. not tethered and with as flexible wiring as possible, are preferred (Liu et al., 1999, Biran et al., 2007). Another factor, assumed to contribute to reactive gliosos, is micromotion resulting from rotational head movements of the subject and the mechanical mismatch between stiff implants and soft tissue (Lee et al., 2005, Subbaroyan et al., 2005). It is hypothesised that implants more mechanically similar to surrounding tissue will experience less motion relative to tissue, giving less strain induced injury and thereby attenuated glial scar. This has already been shown to be an effective strategy in the peripheral nervous system (Lawrence et al., 2002), and theoretical predictions from finite element modelling show that substrates with Young’s modulus closer to brain tissue reduces micromotion related strain by several orders of magnitude (Subbaroyan et al., 2005). Furthermore, tissue attaching to probes (experimentally shown to be primarily astrocytes, McConnel et al., 2007), rather than the non-adherent capsules seen after longer implantation times (> 6 weeks, Turner et al., 1999), have in the same finite element models, been shown to stabilise the implant and reduce strain. Although detrimental to signal transduction, a modest and adhering glial scar might therefore be able to protect surrounding tissue from further injury.

It is at present not clear whether stable probe neuron integration at an early stage can be one way to mitigate glial encapsulation, or at least make neurons part of the glial scar tissue. Numerous studies aim to biochemically either encourage neurons to grow towards the electrode (Winter et al., 2007), attenuate glial attachment and proliferation on the implant (Kam et al., 1999, Azemi et al., 2008), or reduce inflammatory response and thereby save neurons (Zhong and Bellamkonda, 2007, Spataro et al., 2005). It is suggested that if such integration can be obtained at an early stage, this might modulate the development of the surrounding scar tissue to contain more neurons and/or a thinner glial scar (Zhong and Bellamkonda, 2007).

It is evident from the litterature that there is still a need to dive deeper into the biochemical mechanisms of the glial scar. Many pieces in the puzzle considering its evolvement, how it can be modulated and its effect on neighbouring neurons are missing, and a profound understanding of these mechanisms is needed to optimise cortical electrode/neuron interfaces. This will be an important piece of the puzzle to make working long term stable recording and stimulating devices with high selectivity possible.
7 CHARGE INJECTION, FOREIGN BODY RESPONSE AND MICROSIONIZED DEVICES

7.1 Size and charge injection trade off
Reversible charge injection can to some extent be achieved through using state-of-the-art materials like Iridium Oxide, but miniaturisation of electrodes lowers their charge limit proportionally and charge delivery is therefore restricted further with decreasing size. It is important to put all these numbers in relation to each other and sort out what they actually mean for the feasibility of single devices. A review of some of the electrode geometries discussed in Chapter 4 gives a brief insight on how small electrodes need to be, and a review of Chapter 5, how well the materials used today corresponds to the desired charge injection. The Utah Electrode Array does for example have an exposed surface area of around $8 \times 10^{-5}$ cm$^2$. The tips are therefore able to deliver a maximum of 8 nC and 80 nC safe and reversibly if coated with platinum or iridium oxide respectively, and under condition that we adopt the more conservative safe charge injection limits of ~100 µC/cm$^2$ for platinum and ~1000 µC/cm$^2$ for iridium oxide. Conversely, experimental data report stimulation thresholds for cortical activation to be in the range 3-9 nC. The corresponding minimal electrode diameter, assuming circular electrodes, would then be 62-110 µm or 20-34 µm if made of platinum or iridium respectively. If this will be sufficiently small of course depend on the chosen application. For deep brain stimulation only few electrodes (~10-20) are used and miniaturisation and selectivity is not crucial for the application. On the other hand if visual prosthetics is considered, the size of the electrode ultimately decides how much information that can be delivered per unit area, and although the visual cortex provides a rather wide surface (electrodes have to be spaced ~0.5 mm to produce separates phosphenes, Tehovnik et al, 2005) the corresponding surface on the retina poses serious restrictions on the number and size of electrodes that can be used.

7.2 Foreign body response and its influence on charge and size
Grey matter resistivity is reported in literature to be in the range 350 Ωcm at 50 Hz (Latikka et al, 2001). If the foreign body response build up a 50 µm resistive layer of 600 Ωcm (reported to be frequency independent, Grill and Mortimer, 1994), the surrounding resistance is almost doubled. This is of course very simplified considering it will also depend on electrode configuration, stimulation frequency and the amount of fluid in space between the electrode and brain. Not only size but also shape of the electric field will be distorted due to the insulating capsule (Yousif et al, 2008). Nevertheless, it will impact the signal experienced by neurons on the opposite side of the glial scar. A modelling study of similar scar tissue around deep brain stimulation implants predicts an effective increase of 70-100 % of the stimulating current to reach the same level of excitation (Yousif et al, 2008). Mushahwar et al (2007) report a doubling in threshold due to electrode encapsulation, in order to evoke the same response in spinal cord stimulation, from initial experiments and after the first month. If, in addition to this, neurons in proximity to the electrode die or withdraws several hundreds of microns, voltage will need to be raised further to deliver the same stimuli to the same volume of neurons. If thresholds over time need to be doubled, presumably even tripled, due to neurons withdrawing or dying, diameters would need to be in the range 110-
190 µm or 34-59 µm instead (tripled charge delivery). For stimulation purposes at least the possibility to increase electrode size, and thereby the delivered charge, exist. For recording electrodes, on the other hand, experimental experience often shows that neuron signal is completely extinguished over time. The drawbacks of the foreign body response are obvious. Cell communication over fibrotic encapsulation is like speaking through a closed door. Through raising the voice, it is possible to make oneself heard although exhausting and at the risks of missing out on more subtle details in the information. If the people on the other side are still speaking at normal voice and also move away from the door eventually, the communication will with time be increasingly difficult and after a while more or less one way.

![Figure 15: Charge delivery per phase reported in literature for different applications, and the corresponding minimum electrode diameter if electrode material is iridium oxide or platinum and maximum safe charge injection is assumed to be 1 mC/cm² and 0.1 mC/cm² respectively. Reviewed papers are listed in Table 2. * means that charge value was derived from pulse amplitude × pulse width, ** means that the electrode used was interfascicular.](image-url)
To put the charge-size trade-off into context, data collected from the literature reviewed in this report (Table 2) have been compiled in a diagram. In Figure 15 charge density for some common applications are plotted on the same axis and compared to the minimal diameter needed of an iridium oxide or a platinum electrode to deliver that charge. The wide range of animal models, target tissues, electrode configurations and charge densities used, are of course a major source of error, but simplified and for giving at least some figures of merits of the threshold range, this is for the moment disregarded.

Table 2: Papers reviewed to provide data shown in Figure 15.

<table>
<thead>
<tr>
<th>Category</th>
<th>Reference</th>
<th>Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epiretinal stimulation</td>
<td>Humayun et al, 1999</td>
<td>RP or AMD, human</td>
</tr>
<tr>
<td></td>
<td>Jensen et al, 2003</td>
<td>Isolated chicken retina</td>
</tr>
<tr>
<td></td>
<td>Humayun et al, 2003</td>
<td>RP, human</td>
</tr>
<tr>
<td>Subretinal stimulation</td>
<td>Jensen and Rizzo, 2006</td>
<td>Isolated rabbit retina</td>
</tr>
<tr>
<td></td>
<td>Yamauchi et al, 2005</td>
<td>Rabbit retina</td>
</tr>
<tr>
<td></td>
<td>Stett et al, 2000</td>
<td>Isolated chicken retina</td>
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<tr>
<td>Cortical stimulation</td>
<td>Normann et al, 1999</td>
<td>Auditory cortex, cat</td>
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<td></td>
<td>Schmidt et al, 1996</td>
<td>Visual cortex, human</td>
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<tr>
<td>Cuff electrodes, PN</td>
<td>Rodriguez et al, 2000</td>
<td>Sciatic nerve, rat</td>
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<td></td>
<td>Veraart et al, 1998</td>
<td>Optic nerve, human</td>
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<td></td>
<td>Polasek et al, 2005</td>
<td>Peripheral nerves, human</td>
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<tr>
<td>Spinal cord stimulation</td>
<td>Mushahwar et al, 2007</td>
<td>Spinal cord, cat</td>
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<tr>
<td>Intraneural stimulation</td>
<td>Branner et al, 2004</td>
<td>Sciatic nerve, cat</td>
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<td></td>
<td>Branner et al, 2000</td>
<td>Sciatic nerve, cat</td>
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<tr>
<td></td>
<td>Tyler et al, 1997</td>
<td>Sciatic nerve, cat*</td>
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<tr>
<td></td>
<td>Dhillon et al, 2004</td>
<td>Peripheral nerve, human</td>
</tr>
<tr>
<td>Suprachoroidal stimulation</td>
<td>Yamauchi et al, 2005</td>
<td>Rabbit</td>
</tr>
</tbody>
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* = derived from pulse amplitude x pulse width,
RP = Patient with Retinisc Pigmentosa, AMD = Age-related Macular Degeneration
8 BACKGROUND AND METHODS – CONDUCTING POLYMERS FOR NEURAL INTERFACES

8.1 Conjugated polymers – conducting plastics
Conjugated polymers are a family of polymers that, depending on doping, can transition from insulating to semi-conducting and conducting states. This property is a consequence of the electronic structure of the polymer backbone, the conjugation of the polymer. To explain the function behind the conductivity of conjugated polymers, it is instructive to start with a simpler polymer chain than PEDOT. In Figure 16a below the structure of the conjugated polymer trans-polyacetylene can be seen. Polyacetylene is a conjugated polymer in the context of that every second carbon-carbon bond in the polymer backbone is a double bond. The four valence electrons of each carbon atom are sp²-hybridised. Three of the four sp² orbitals spread out at 120 ° angle in the same plane, and the fourth electron forms a pₓ orbital, perpendicular to the others. While the three co-planar orbitals form localised σ-bonds between the carbon atoms in the polymer chain and to hydrogen; electrons in the pₓ orbital interact with pₓ electrons on neighbouring carbons to form another type of bond. These bonds are called π-bonds, and are more delocalised than the σ-bonds.

Electrons in these delocalised bonds can be compared to the particle-in-a-box model well known from quantum physics. The particle in a box can transition between different energy levels and according to the Pauli principle, each level can host two particles of opposite spin. In its pristine state, N carbons of polyacetylene host N electrons in the π-bonds. If each carbon unit comprise a one-dimensional box for the π-electron, there should therefore be room for an additional N electrons to be inserted into the π-bond at the same energy level at each carbon atom. The un-doped form of polyacetylene would have a half-filled energy band and be expected to have metallic conduction properties. However, in reality we know conjugated polymers to be semi conductors in their un-doped state, which indicates that there should be a band gap to the next unoccupied electron state. This band gap is the result of a conformation change, from the equidistantly spaced carbon atoms, to an alternating structure of longer and shorter bonds. Therefore, instead of having a polymer chain consisting of equivalent single-carbon units, two carbons form the smallest unit cell of the polymer. This in turn means that there are already in the un-doped state two π-electrons occupying the lowest energy level and there is an energy gap to the next unoccupied level. The terms Highest Occupied Molecular Orbital (HOMO) and Lowest Unoccupied Molecular Orbital (LUMO), are often used to describe these energy bands as shown in Figure 16b.

In the completely filled HOMO level π-electrons are, although delocalised, not freely mobile along the polymer backbone. For the polymer to be highly conducting it is therefore essential to introduce charge carriers in the electronic structure. This can be achieved by doping of the polymer, which simplified means withdrawing electrons from the HOMO level or inserting electrons in the LUMO level, leading to a partly filled band and increased conductivity. The process is in reality of a more complex nature. The insertion of holes or electrons in fact
modifies the bond pattern of the polymer and introduces more energy levels within the band gap. Increasing the doping level also increases the number of charge carriers occupying such intermediate energy levels. These charge carriers are not delocalized although they can, if neighbouring unoccupied energy states are available, tunnel from one state to another. Charge carriers can in this manner move along the polymer chain if the doping level is high enough to yield sufficient amount of such intermediate states for tunnelling to occur at high probability. This effect is the origin of the conducting properties of doped conjugated polymers.

Figure 16: a) Polymer structure of trans-polyacetylene. b) HOMO and LUMO levels in a conjugated polymer.

Figure 17: Polymer structure of a) polypyrrole and b) poly(3, 4-ethylene dioxythiophene) (PEDOT).

There exist a wide selection of conjugated polymers that can be doped into highly conducting states. Polypyrrole (PPy) (structure, Figure 17a) is extensively studied for biological applications and comprehensive testing has shown the biocompatibility of PPy as implant material (George et al, 2005, Wang et al, 2004, Cui et al, 2003). Although PPy have until recently been the conjugated polymer of choice for biological applications, the more stable composition of PEDOT (Figure 17b) (Li and Pickup, 2000, Heywang et al, 1992, Pei et al 1994) has led to a shift in focus to the latter over the last ten years.

8.2 PEDOT for neural electrodes – why PEDOT?

From the discussion in Chapter 3-7 it can be seen that there is still a great need for new materials to be developed for neural electrodes. Glial encapsulation and neuronal loss around electrodes increases charge injection requirements and also deteriorates recording capabilities
of the electrode over time (Biran et al, 2005), compromising selective long term performance. Furthermore, for some applications selectivity will be driven by the possibility to deliver more charge reversibly from miniaturised electrodes. To overcome these obstacles, three possible strategies are identified. Better long term performance can be found through either:

1) finding materials that allow more charge to be delivered from smaller electrode surfaces, so charge delivery can be raised to surpass the glial scar,

2) finding strategies to bring neurons closer to the electrodes, i.e. counter acting the glial scar or encouraging neurons to grow towards the electrode.

or

3) finding ways to bring electrodes closer to the neurons, surmounting the glial scar tissue after it has evolved.

Dense capsules might distort the signal and therefore smaller electrodes are no guarantee for higher selectivity. Furthermore, delivery of more charge in tissue also increases the risk of stimulation induced tissue damage and is therefore a strategy that should be applied cautiously. More importantly, strategy 1 does not solve the problems concerning selective recording and therefore only gives half of the solution to the bi-directional interface. Reduction of the glial scar is therefore in many ways the most attractive strategy to solve the problem. Whether some glial scarring can be tolerated remains to be determined for each application. It is possible that some scarring is inevitable and even necessary to protect tissue from the implant. There are also some suggestions on how the probe can infiltrate past the scar as in strategy 3 (Richardson-Burns et al, 2007). On the positive side, PEDOT can be useful in all three of these strategies, as will be explained thoroughly in the remainder of this thesis.

8.3 Tailoring PEDOT for the neural interface

The most important property of polymer materials is that it can be tailored both mechanically, chemically and biochemically to suit a specific application. Through understanding the fundamentals of polymer properties, chemical engineering can be applied, for example, to make polymers conducting as with PEDOT and PPy. Biomolecules can be attached to the polymer chains or mixed in during synthesis to create composite biomolecule/polymer materials. Mechanical properties can be engineered to anything from stiff and hard films to polymer gels, either biodegradable, if required, or long term stable. Additionally, polymeric material is comparably cheap to noble metals and is easy to process and pattern both macroscopically and microscopically.

Ideally, it would be possible to combine all the desired properties into one material, specially designed for neural interfaces. Of course, combining all good properties at once is not easily performed. Alterations of the material to suit one aspect might interact with one of the other properties, and the process will need much iteration before the final goal is reached. It is evident however, that working with polymer materials gives the opportunity to create electrode materials mechanically and chemically more similar to neural tissue than metals,
which indicates that it might also stand better chance of acting as a stable interface with neurons over time.

The mechanical mismatch between silicon and the body has already been pointed out as a contributing factor to more extensive foreign body response. Polymer electrodes can be made with mechanical properties similar to that of actual cells (Section 8.3.3) and it is hypothesised that such soft electrode coatings would act as mechanical buffering between stiff probes and soft tissue. Furthermore, similarities between the chemical structure of PPy, and naturally occurring hemoproteins and pigments like melanin, gives promise for future biocompatibility of conjugated polymers.

The concept of using conducting polymers for the improvement of neural interfaces has already been studied by several groups. Nyberg et al (2002) polymerised PEDOT: poly(styrenesulfonate) (PSS) into hydrogel electrodes and demonstrated its attractive electrochemical properties both for neural recording and stimulation. The authors suggested such PEDOT gel electrodes, applied on poly(di-methylsiloxane) (PDMS) substrates, as the basis for truly soft electrode assemblies in the peripheral nervous system. Although softness of the complete electrode assembly is often sought, silicon microfabricated electrode arrays have a long tradition especially as cortical interfaces. Applying a layer of conducting polymer to improve electrode/tissue interaction on already existing silicon shafts, as described by the Michigan group in numerous studies (Cui et al, 2001, Cui and Martin, 2003), is therefore a useful way to investigate the potential of PEDOT material in a well established experimental setting. Preliminary testing show encouraging results both on the electrochemical and the biocompatibility side (Ludwig et al, 2006, Cui and Zhou, 2007, Cui and Martin, 2003, Nyberg et al, 2007), and the interest for PEDOT as coating material for neural electrodes is therefore continuously increasing.

8.3.1 PEDOT/biomolecular composites

A straightforward and simple way to build adherent and water resistant PEDOT films on conducting substrates is through electropolymerisation. In brief, a solution of monomer and counter ion is prepared. The conducting substrate is connected as working electrode in a three electrode setup, immersed in the monomer (EDOT) solution, and current is driven through the electrochemical cell. As a result, monomers in solution build up polymer chains at the working electrode incorporating the counter ion (CI) in the process through the following reaction:

\[
\text{Eq. 8-1}
\]

The process can be driven either potentiostatically or galvanostatically, and in general the more charge that is applied during the process the more polymer growth is achieved. To avoid solvent related biocompatibility issues it is practical to use aqueous monomer solutions. Unfortunately, the water solubility of EDOT is rather poor, below 0.01 M, and as a consequence surfactants are often suggested as counter ions to mediate EDOT solubility. Common surfactants are polystyrene sulphonate (PSS) and docedylbenzenesulphonate (DBS).
and it has been shown that their presence facilitates electropolymerisation from aqueous solutions (Bobacka et al., 2000, Lima et al., 1998, Sakmeche et al., 1999).

Although the use of surfactants like PSS is strongly motivated from the electrochemical standpoint, there might be cons to this approach from the biomaterials point of view. Apart from its surfactant properties PSS is also an acid (pKa = 1). Although preliminary studies have shown that cells can proliferate and grow on top of PEDOT:polystyrene sulphonate (PSS) (Isaksson et al., 2007, Cui and Martin, 2003) and pH in the body is tightly regulated, possible negative effects of introducing acidic surfactants like PSS, in delicate tissue, raises the question if it can be replaced by something more trustworthy? This is especially important keeping in mind the ambition of not just ensuring that material is not directly toxic to cells, but preferably should encourage cells to grow close to or even into the material. In fact, the incorporation of the counter ion in the polymer is an excellent opportunity to build in something that can mediate the foreign body response, encourage neuron ingrowth, discourage astrocyte adhesion and suppress inflammatory response.

8.3.2 Electrochemical performance of PEDOT/biomolecular composites

The aim of Paper I of the present thesis is to investigate whether electrochemical polymerisation from aqueous solution is possible using different biomolecular ions as counter ion in the electropolymatisation of PEDOT, completely excluding the use of surfactants. A small selection of biomolecules; heparin, fibrinogen and hyaluronic acid were chosen based on their natural presence in tissues and their commercial availability in larger quantities. Heparin is a potent anticoagulant which can be used to increase surface haemocompatibility and hydrophilicity, and can also be used for the binding of important growth factors, for instance neural adhesion molecule N-CAM, to the material (Cole and Glaser, 1986, Sakiyama et al., 1999). Fibrinogen is a fibrillar protein that is central for the mechanism of coagulation and thrombosis. Polymerised fibrinogen form fibrin matrices with an important role both in the spontaneous wound healing response and as man made tissue engineering scaffolds. Numerous reports point out the ability of fibrin gels to form scaffolds that enhance nerve regeneration in the peripheral nervous system (Sakiyama et al., 1999, Herbert et al., 1998), and it is therefore not far fetched that it would be interesting to form conducting polymer/fibrinogen scaffolds at electrodes aimed at neural integration. Hyaluronic acid (HA) is a glucosaminoglycan and an important component of the extracellular matrix also in neural tissue. HA forms viscoelastic gels even in very dilute water solutions and is therefore used in medical applications such as supplementing the lubrication in arthritic joints and for targeted drug delivery. It is often suggested as a suitable tissue engineering scaffold and several authors report its ability to improve peripheral nerve regeneration (Wei et al., 2007, Wang et al., 1998, Özgenzel, 2003).

Paper I describes the electropolymerisation of PEDOT, using these three biomolecules as counter ions, from aqueous solutions and without the use of any surfactants. Electrochemical methods, including cyclic voltammetry (CV) and impedance spectroscopy, are applied to investigate how the incorporation of the biomolecule changes electrochemical and surface properties, and measurements are compared to the extensively used PEDOT:PSS.
8.3.3 PEDOT hydrogel electrodes

Usually, polymer films formed by electrochemical deposition build thin and dense polymer films with thickness proportional to the deposition charge. Films produced in this manner have a rough microporous surface but also the bulk of the polymer film is permeable to the electrolyte. One factor determining the extent of this permeability is the counter ion used (Paper I, Bobacka et al 2000), but porosity can also be tuned by other means in the polymerisation process. Ghosh and Inganäs (1999) showed that through applying a film of ionically cross-linked PEDOT:PSS dispersion onto the electrode prior to polymerisation, allowing electropolymerised material to grow into the loosely bound polymer networks, the resulting polymer film had superior charge storage and impedance properties compared to the normal electropolymerised films. They found that the reason for this effect was that the permeability of the polymer to the electrolyte was greatly improved. Hence, the contact area of the polymer chains with ions in the electrolyte was increased, thereby enlarging the electrochemically effective area of the electrode. The authors used hydrogels made of PEDOT:PSS dispersion and bivalent ions, but in fact this method is not restricted to conducting gels. Subsequent studies (Kim et al, 2000, Kim et al, 2004) show that conducting polymers can grow through non conducting scaffold gels as well, under the condition that the gel porosity is sufficient to allow polymer ingrowth and the gel is adherent to the electrode surface (Figure 18).

Besides enhancing the electrochemical area of the electrode, the method creates softer electrodes than the more densely packed polymer structure built without the application of a gel scaffold. It is hypothesised that the softness created by a conducting gel layer will help reduce the effect of the mechanical mismatch between substrate and neural tissue. The porous hydrogel layer could presumably also be a more attractive surface for neurons to grow into, and modelling studies show that better probe adhesion can reduce effects of micromotion (Lee et al, 2005, Subbaroyan et al, 2005). Hence, hydrogel electrodes are a possible way to tailor the mechanical properties as well as the electrochemical properties of PEDOT. This thesis does not cover experimental work on such hydrogel electrodes apart from the inclusion of an MgSO₄ cross-linked version of the PEDOT:heparin surfaces investigated in the in vitro biocompatibility study of Paper II. However, the PEDOT/biomolecular composites studied here could presumably be grown into hydrogel scaffolds as well, further improving their electrochemical performance.

Figure 18: Polymer grown electrochemically through a NaAlginate hydrogel network.
8.4 PEDOT as a biomaterial – new challenges with new materials

8.4.1 *In vitro* and *in vivo* biocompatibility testing of PEDOT/biomolecular composites

Although preliminary studies have shown very promising results on the potential of PEDOT to significantly improve both recording and stimulation properties of the neural interface, these advantages do presumably come at the cost of less experience of the long term performance of these materials *in vivo* compared to already established biomaterials like platinum. In Paper II in this thesis two methods, described in ISO 10993-5, are applied to evaluate cytotoxicity of the PEDOT:biomolecular composites introduced in Paper I. Non-direct contact tests, an agarose overlay assay and an elution assay, are applied to separate the question of cell preference for growth and proliferation on the material surface from pure cytotoxicity of material components. Material toxicity is evaluated using two well established cell lines; the L929 mouse fibroblast cell line suggested in the ISO document, and for testing with more neuron like cells, the human neuroblastoma cell line SH-SY5Y. Cell viability was examined through staining with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) which is reduced to purple formazan in the mitochondria of living cells. Cells were analysed macroscopically, a toxic material will produce a clear zone of decoloration underneath the sample, and microscopically, to reveal any morphological changes. Cell response was compared to that of a known toxic control material and to the assumed non toxic gold on glass substrates used for polymerisation.

The incorporation of biomolecules in the material does not only provide a way to build polymer electrodes without the interference of surfactants, but also introduces species which have a specific purpose in the living system. It is therefore important to study the effects of the material and its interaction with tissue *in situ* and in the presence of the immune system. From the cytotoxicity evaluation in Paper II, and the electrochemical evaluation in Paper I, PEDOT:heparin was determined to be the best candidate for neural electrodes and was, in order to study systemic response, implanted in the cortex of Sprague-Dawley rats (Paper II). Cortical tissue was examined histologically after three weeks and six weeks, and compared to controls which had been implanted with platinum probes of equal size and shape. The implants were not used for stimulation or recording but only response to the passive implant was evaluated.

8.5 PEDOT stability – *in vitro* evaluation of *in vivo* stability

Although PEDOT is reported to be the most stable electrically conducting polymer available (Li and Pickup, 2000), it is crucial that materials intended for implants are stable over long periods of time. First, the long term benefits of the implant must be of substantial impact to motivate the risk taken by the surgical procedure. Second, in case of degradation of the implant, degradation products might in them selves be harmful although the original implant is proven biocompatible. The stability of PEDOT as implant material has yet not been proven. Therefore Paper III in this thesis presents an *in vitro* evaluation of the expected stability of PEDOT in biological environments. With respect to the increasing interest in replacing well established surfactant counter ions for biologically more relevant counterparts (Paper I, Cui *et al*, 2001, Kim *et al*, 2007), it is important to study how this might influence
not only electrochemical properties short term, but also if the long term stability of PEDOT electroactivity is altered by this replacement.

To allow for precise control of different environments and their effect on material over time through measurements at regular time points, an *in vitro* simulation of the biological environment was used. PEDOT coated surfaces were immersed in two solutions, either 0.01 M phosphate buffered saline (PBS) or 0.01 M H$_2$O$_2$, at 37 °C, to simulate exposure of implant to living tissue. Electroactivity of the coating was probed with CV, and optical changes were studied with transmission spectroscopy in the visible range. Measurements were performed every second to third day, throughout a six weeks period or, until optical absorbance or electroactivity was completely lost.

Distilled water (37 °C) was used as control environment and apart from control samples, exposed to same type of measurements as the probes in H$_2$O$_2$ and PBS, some additional samples were also stored in distilled water throughout the study and without being exposed to measurements. These samples were only measured at the beginning and the end of the study period, and were used to exclude the possibility of additional material degradation through the repeated measurements. Furthermore, to study the influence of voltage pulsing on stability, a group of samples were also exposed to biphasic pulsing (-0.2 and 0.2 V vs. Ag:AgCl) in PBS continuously between measurements. These probes were however not followed for the whole six weeks period, but only for a shorter period of 11 days.

The concentration of H$_2$O$_2$ at 0.010 M is estimated to be roughly 10 times the physiologically relevant concentration, meaning that the protocol applied is an accelerated degradation. Direct translation on how increased concentration corresponds to longer time, is not straightforward to make. However, the study period is rather short compared to the long term implantations neural electrodes are intended for, and an accelerated process giving information about performance beyond the six weeks was therefore considered necessary.

### 8.6 PEDOT woven electronics – new directions with new materials

#### 8.6.1 The ideal electrode array – a textile structure?

The success of an implant does not solely depend on biomaterial aspects on the surface, but also the foreign body response to the complete device must be taken into consideration. A general description of the cortical electrode array is a flexible and porous structure with a “high” number (10-1000) of small active electrode surfaces that each can be individually addressed. A traditional method to implement such a structure is through planar patterning techniques like photolithography, and semiconductor technology on silicon wafers. As already discussed thoroughly in this thesis, these structures are, although practical to fabricate and handle, not necessarily the best choice for creating implantable nerve interfaces. It is even possible that such stiff and compact components will, despite of their increased complexity, never be able to successfully communicate with neurons long term. Even though sophisticated coatings might suppress glial encapsulation and encourage neuron survival around the implant, it will be difficult to deliver such control molecules continuously, and the persistence of the array will still elicit a foreign body response intended to either dissolve and remove, or encapsulate, the foreign body. It is therefore interesting to investigate alternatives
to these compact electrode assemblies, and especially if the introduction of PEDOT to this field can be used in other ways that just as coating, tentatively to build different kinds of electrode arrays.

Observations of foreign body response to structures with subcellular dimensions have indicated that below a certain size cells can not attach to the object, meaning that the mechanotransduction needed to mediate the response to the foreign object is not initiated (Seymour and Kipke, 2007, Stice et al, 2007, Sanders et al, 2000). Simplified, it can be said that a sufficiently small structure holds some degree of immunological invisibility or at least is not treated in the same way as a bulkier foreign object. If the object is truly subcellular, like a small bead, it most probably would be phagocytised. If instead a thin (<10 µm) but long structure, like a thin fibre, is implanted, the object can not be phagocytised but also not encapsulated through the same mechanism as a larger object. Combining the observations of immunological “invisibility” of thin structures with the quest for a flexible implant withholding a large amount of individual conductors, it is not far-fetched to think of a textile.

Textiles, meshes or weaves have been extensively used in the medical field, e.g. as implants, due to their flexibility and their open structure which allows tissue ingrowth and vascularisation. In a textile individual threads are not bound together, but slides freely over each other, which macroscopically give the weave elastic properties even when the individual fibre components are not flexible. These properties make weaves ideal structural components in tissue engineering as well as cardiovascular stents. Thousands of years of textile development also provide an almost endless versatility of fabrication techniques, and although two dimensional weaving is undoubtedly the most common, certain methods can produce three dimensional meshes of virtually any shape and structure. Monofilaments in the diameter range of 10 µm are not unusual in textile production, and even small scale handicraft techniques can be used to produce three dimensional structures from fibres in this size range. For example, the three-dimensional lace in Figure 19 is made from cupper wire using bobbin lace technique.

It has already been shown that conducting polymers can be coated onto fibres to form conducting threads. Furthermore, polymerisation on non-conducting textiles have been used to create textile sensors with applications for sports and rehabilitation medicine (Hong et al, 2005, Wu et al, 2005, Li et al, 2005). Single conducting fibres could hence be used as both construction material and as signal transduction wires in the electrode array. It is possible to implement this idea without the use of conducting polymers, for instance, using thin and metallised fibres with an insulated surface. This approach would be limited by the fact that every electrode terminal would need a complete individual conductor for signal control and, with increasing electrode densities, the amount of wires would soon be unmanageable. The use of conducting polymers does however introduce an alternative approach to solve this problem.
8.6.2 The PEDOT electrochemical transistor

PEDOT is a semi conducting material, meaning that it can be switched between highly conducting (oxidised) and low-conducting (reduced) states through doping and dedoping of the polymer backbone. The conductivity change can be up to 5 orders of magnitude (Johansson et al., 2002), an effect which can be used to create transistor function (Nilsson et al., 2005). The basis of such PEDOT based transistor components is the interaction between two adjacent PEDOT structures over an electrolyte (Figure 20a).

Consider the following example and the transistor sketched in Figure 20a:
At time point 1, PEDOT is in its oxidised, conducting state. Voltage is applied over drain to source (VDS), and constant current (IDS) flows accordingly. At time point 2, voltage is applied over the gate contacts (VG), and interaction between G1 and G2 lead to a reduction of the film covered by the electrolyte into its low-conducting state. Hence, a resistive channel forms, suppressing the drain-source current flow. In short, through applying a voltage over the gate contacts the current flow from drain to source can be controlled. An example of measured drain-source currents for different values of VG can be seen in Figure 21. Such electrochemical transitors (ECTs) have been the basis for the development of a wide range of printable thin-film electronic components thoroughly investigated at the University of Linköping.
8.6.3 The Wire Electrochemical Transistor – and its potential for the woven multi-electrode array

The electrochemical transistor performance, measured in on/off ratio, is best when relatively thin films are used. However, its geometry is not necessarily restricted to thin planar films, but could just as well be extended to thin films on cylindrical substrates as shown in Figure 20b. If the cylindrical substrate is an insulator, current along the cylinder can only flow in the PEDOT film coating. Voltage applied over the gate cylinder reduces the film covered by the spherical electrolyte and breaks source drain conduction just as for the planar structure. In fact, the transistor characteristics in Figure 21 are measured on such a cylindrical ECT. This discovery was first published by Hamedi et al (2007), and also gave rise to a patent application (WO/2008/066458) considering how the implementation of the electrochemical transistor in cylindrical configuration can be used to build transistors in textile structures. The transistor type was denoted Wire Electrochemical Transistor (WECT) and it was concluded that it could be used to build in logic circuits in textiles. Paper IV in this thesis deals with the question on how the WECTs can be useful for the implementation of woven electrode arrays. Especially, addressing circuits are considered, since such circuits will be central for the implementation of the high electrode densities that will be needed in future neuroprosthetics. The focus of Paper IV is conceptual, and simulations modelling experimental WECT data to show the feasibility of the suggested designs are included. Some preliminary experimental work on PDMS encapsulation of WECT circuits is also described in this paper.

Figure 20: PEDOT electrochemical transistors. a) A planar version of the electrochemical transistor. Voltage applied over the gate contacts (G1 and G2) can reduce the PEDOT into a non conducting stage, thereby breaking connection over the drain source contacts (D and S in figure). b) Cylindrical version of the same transistor.
Figure 21: Transistor characteristics for the cylindrical electrochemical transistor. Increasing gate voltage lead to a decrease in drain source current. Gate voltages are, on the right, listed in V.
9 RESULTS - CONDUCTING POLYMERS FOR NEURAL INTERFACES

9.1 PEDOT/biomolecular composites – experimental results

The electrochemical experiments in Paper I clearly show that electropolymerisation of PEDOT with heparin and HA is straightforward and a possible way to functionalize PEDOT. The electrochemical properties of formed PEDOT:heparin films were similar to PEDOT:PSS, which is interesting considering that no surfactants were used in the electropolymerisation of the former. PEDOT:HA was not found to be as electroactive as the PEDOT:PSS and PEDOT:heparin films, but still properties were found to be in the range needed for neural stimulation and recording electrodes, and impedance of the surface was, as expected (Cui and Martin, 2003, Nyberg et al, 2002), lowered several orders of magnitude from the bare platinum surface.

PEDOT:fibrinogen films were difficult to deposit through electropolymerisation, since the affinity of the fibrinogen to the platinum surface led to insulating adsorbed layers of protein blocking the surface before a polymerisation reaction was established. Although PEDOT:fibrinogen films could be formed reproducibly through a modification of the polymerisation procedure, these films were low conducting compared to the other films. Despite an extremely porous polymer surface of electropolymerised PEDOT:fibrinogen, as revealed by Scanning Electron Microscopy, it was concluded that adsorbed non conducting elements blocked electrolyte contact with the conducting elements in the film, and hence electroactivity of the formed polymer was comparably poor.

A comparison between the responses to a cathodic pulse of 0.5 V, for films formed with the different biomolecular counter ions, can be seen in Figure 22. 0.5 V is estimated to be well below the voltage limits where over-oxidation of the film takes place. The pulses used in Paper I (50 ms) are longer than necessary considering that myelinated nerve fibres have chronaxies in the range 0.4-0.7 ms (Mogyoros et al, 1996), and reportings of suitable pulse widths for visual cortex (Schmidt et al, 1996) and optic nerve (Veraart et al, 1998) induced phosphenes are in the range 400-600 µs and 100-400 µs respectively. This means that the charge deliveries reported in Paper I is an overestimation. The sample times used in the measurements do not allow an exact prediction on how much charge that can be delivered during the initial 600 µs of the pulse, but linear regression from measurements on the first two milliseconds of the pulse can be used to answer this question and yields charge deliveries as follows; PEDOT:PSS, 47 µC/cm², PEDOT:heparin, 43 µC/cm², PEDOT:HA, 43 µC/cm² and PEDOT:Fibrinogen, 23 µC/cm². This approach only gives a rough estimate of the expected charge delivery for such short pulses. On one hand, a slower response of the polymer electrode would make this value an over estimation; on the other hand it is likely that the actual peak current is higher than the sampled peak value, and hence this calculation would yield an under estimation.

Although the charge deliveries might seem modest compared to the 100 µC/cm² reported for platinum (Rose and Robblee, 1990), one should keep in mind that these electrodes were not driven to the absolute potential limit, but 0.2 V is well below the maximum limit where
over-oxidation occurs. Furthermore, since the hydrogel electrode procedure (Ghosh and Inganäs, 1999, Nyberg et al., 2002) was not included, material charge injection could be boosted further with such techniques. The impedance spectra, in Figure 23, for a platinum electrode with and without a film of PEDOT:PSS (deposition charge, 0.6 mC/cm$^2$) shows a clear drop in impedance over the whole frequency range, meaning that the same potential from a polymer coated surface, compared to that of a pure platinum surface, will deliver significantly higher charges from the former. Hence, deposition of a PEDOT film on the platinum surface is a major improvement in this aspect. Although no surfactants were used for the PEDOT/biomolecular composites, the electrochemical properties of the films were similar, or close to similar, to that of PEDOT:PSS, especially for HA and heparin.

![Figure 22: Current response to an applied cathodic pulse of -0.5 V for 50 ms, for the different PEDOT:biomolecular composites described in Paper I and II.](image)

![Figure 23: Impedance spectra of a bare platinum electrode, compared to that of a PEDOT:PSS coated platinum electrode, in KCl electrolyte.](image)
In vitro toxicity studies (Paper II) also showed non cytotoxicity for all of the PEDOT materials indicating good biocompatibility of the formed polymers, and that no residual toxic monomers or other toxic traces were left in the PEDOT films. Histological evaluation of PEDOT:heparin in SD rat cortex showed no marked differences between tissue exposed to PEDOT:heparin coated platinum probes compared to pure platinum implants. This is a good indication both on the biocompatibility of the PEDOT material and that the inclusion of heparin in such implants does not on-set any undesired immunological reaction.

A problematic issue in the in vivo biocompatibility study (Paper II) was however the substantial glial encapsulation seen. This is expected, considering that this foreign body response is known from literature (Chapter 6) and the platinum foil used as substrate is comparably thick and stiff. The variability between subjects was also large and it is possible that more subtle reactions to the PEDOT:heparin material would be masked by the strong astrocytic response. This is a weakness of Paper II that can not be disregarded, and although data presented showed no statistically significant differences between the two implant types, it would be interesting to repeat the study with an implant design yielding less encapsulation and also less variability within groups. For instance, a stereotactic frame could be used for the surgery and microfabrication could be applied to make thinner implants with high precision. Another important issue that should be addressed with future biocompatibility studies is if the application of a stimulus potential will influence cellular response to the material. It is possible that a passive probe does not reveal the complete response to the material when used actively as an electrode for stimulation.

One of the questions raised in this thesis, and also explicitly requested in a recently published paper by Green et al (2008), is the stability of PEDOT as an implant. Green et al specifically pointed out the importance of clarifying the impact of adding biological components on the polymer properties and the “changes polymer will undergo in vivo”. The stability study in Paper III can at least partly answer this question. From Figure 24 the decline in electroactivity (i.e. cathodic sweep area) over time, measured in percentage of original electroactivity, can be seen. Each line represents one of the material samples and lines with markers represent samples exposed to H$_2$O$_2$, whereas unmarked lines represent samples exposed to PBS. Statistical calculations on electroactivity decline in different solutions did show significant$^1$ differences in decline rate, and also the time course of the decline separated the two materials. The decrease for PEDOT:heparin appears to be more linear compared to PEDOT:PSS, which is initially less affected (< 7 days) but then falls more steeply in electroactivity.

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$^1$ ANOVA, on samples exposed to PBS, distilled water and PBS + voltage load p<0.05
More importantly, the steep decline of PEDOT electroactivity for both materials show that PEDOT is sensitive to oxidising agents also in the dilute concentrations used here. Over-oxidation of PEDOT breaks the conjugation within the polymer and thereby the electronic conductivity, and is consistent with the findings of Paper III. It should be noted that the H$_2$O$_2$ concentration used in the stability study presented here is 10 times the expected physiological concentration, which mean acceleration compared to the degradation that would be expected in vivo. The real survival in vivo will be influenced by the extent of attack from the immunological system as well as other factors like protein adsorption to the surface as explained for platinum in Chapter 5. Whether these factors put together will increase degradation, or counter act to protect the material, remains to be determined. Observations of the PEDOT:heparin probes explanted after 6 weeks in rat cortex revealed that, at this stage, the films were still the characteristic blue shade of PEDOT, which is a good indication of retained film conductivity. Loss of electroactivity will correlate with loss of optical properties as well. These observations, together with the fact that the concentration of H$_2$O$_2$ was tenfold the expected physiological concentration, support that material survival in vivo will be longer than six weeks. Exactly how long must be evaluated in proper in vivo experiments. It is however clear that, in the presence of an oxidising agent, PEDOT will over-oxidise and loose its attractive electrochemical properties over time, a fact that must be dealt with when long term applications are considered.

Another important observation of Paper III was that depending on the substrate (ITO or gold) delamination of films was a significant problem. In fact, nearly all PEDOT films, regardless of counter ion, delaminated from an ITO surface within days when immersed in PBS. We hypothesise that this is due to swelling of the polymer film, not matched by the substrate, and the particularly smooth ITO surface providing poor film adhesion compared to gold. These results points out that the substrate used will have significant impact on film adhesion, and if additional abrasion is added, both on insertion of the implant in tissue and from continued micromotion, this might be an even more serious threat to electrode stability than over-oxidation.

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9.2 PEDOT woven electronics – results of conceptual study

In Paper IV the possibility to use a new type of woven logic (WECT) to build electrical interfaces with the nervous system was investigated, both considering circuit design of the woven electronics for addressable electrode arrays and through simulation of such circuits in the analogue circuit simulator SPICE. A SPICE predefined PMOS depletion mode transistor was customised to fit the characteristics of the WECT, and effort was made to account for the slower switching time of the electrochemical transistor. The suggested woven circuits were primarily intended for addressing bi-phasic pulses through the network, thereby directing the pulse to a specific electrode terminal. Circuits were not aimed for producing such bi-phasic stimulation pulses, which would be impossible using only the slow switch of the electrochemical transistor.

The most important circuit discussed in the paper is the three-dimensional addressing structure, intended to address pulses to specific electrodes in a matrix based on a two component address (x and y) as shown in Figure 25. The pulses should be delivered to wires extending in the z-direction (Figure 25a) based on addressing signals in the x-y-plane. Electronically the matrix assembly could be implemented through parallel coupling of two transistors taking the x and y address signals as their respective gates, as shown in the circuit layout in Figure 25b. Extending the weave into two more planes, one ground-connected and one signal bearing and interconnected over the address plane as shown in Figure 25c, the required stimulation matrix would be complete. Stimulation electrodes are thereby kept at ground potential until transistor channel is closed by simultaneous addressing of both transistor gates. The circuit layout can be implemented in a woven structure if one makes use of the fact that different layers in the weave can be coupled in three dimensions as shown in Figure 26 (not shown in Paper IV). The yellow spheres indicate electrolyte positions and the
yellow crosses indicate short circuits between fibres. Complete ground and signal bearing planes are for clarity not included in the weave layout.

Figure 25: Electronic layout of a WECT system for directing bi-phasic stimulation pulses through a three-dimensional network. a) x-y-addresses are used to direct a pulse to one specific electrode, sending current up in the z-direction, b) transistor coupling to implement this system, where x-signal and y-signal are used as transistor gates, and their simultaneous addressing, breaks connection to the ground plane, thereby sending the bi-phasic stimulation up in the z-direction. c) The three-dimensional layout of many such circuits, where the input signal, and the ground connection in b), are extended to whole planes.

The results of Paper IV, together with results presented by Hamedi et al (2007), clearly show that theoretically it is indeed possible to implement such circuits using woven logic and fibre diameters in the subcellular size range. The results of Paper IV also point out some practical concerns both considering the fibre coatings, and the transistor sensitivity to dehydration due to the electrolyte based switching. One further development needed, also mentioned in the paper, would be fibre terminals aimed at charge delivery to the tissue interface. This could presumably be done through the application of a hollow fibre, filled with polymer, connecting to the PEDOT coat of the signal bearing fibre. This PEDOT filling could, for example, be a PEDOT gel electrode, either in electropolymerised form or from deposited PEDOT:PSS dispersion (Figure 27, not shown in paper).
Figure 26: Woven layout of the transistor coupling in Figure 25.

Figure 27: Connection of hollow insulating fibre, with PEDOT filling, to signal bearing fibre from the woven logic.
10 EPIDEMIOLOGY OF TRAUMATIC NEURAL INJURY AND AMPUTATIONS

10.1 Peripheral nerve injury and amputations

This thesis is focused on the topic of material development aimed at expanding the number of neuroprosthetic applications through the construction of better implanted electrodes. Although some of the questions concerning neuroprosthetic electrodes are general, there is also a need to move on from the general discussion and target these techniques for specific applications. The choice of primary targets must naturally be based on the technical feasibility of such applications, but should also be made with an understanding of the epidemiology of these injuries to identify areas where neuroprosthetics can contribute the most.

Originally, this thesis was mainly aimed at solutions for the peripheral nervous system, such as neural engineering for the healing of peripheral nerves and nerve interfaces for artificial feedback or neural control of prosthetic limbs. Therefore, an epidemiologic study was conducted with the aim of clarifying the epidemiology of such injuries. Thanks to a long history of routine collection of health care information in Sweden, detailed data on such injuries are available, and an overview of the epidemiology can be obtained. The Swedish hospital discharge register (HDR) documents all public in-patient care in Sweden, and data was collected for all relevant injuries, between the years 2002 to 2007, for the analysis in Paper V.

It is important to understand that nerve injury is a general term involving everything from a slight stretch of a peripheral fibre to the complex and often devastating brain damage. While slighter peripheral nerve injuries can heal well providing full recovery of function for the patient, more severe injuries show poor recovery and can be the cause of lifelong disability. Since HDR at present does not contain data considering severity of injury or outcome of treatment, total time spent in in-patient care unit per injury was used as a proxy for socio economic cost and an indirect measure of injury severity. The incidence of selected codes and their causing factors, age and gender distribution, was discussed in detail. The following three criteria were used to screen the material, and an injury was singled out as potential target if criterion 1, and at least one of criteria 2-3 below, were fulfilled.

1) The mean in-patient care time for treatment of this injury was more than a week

2) At least 20 patients per year were submitted to Swedish hospitals with this diagnosis

3) The total amount of in-patient care days spent on treatment of such injuries per year was more than 100
10.2 Epidemiology – Results of work

In total, during the nine year period, 11 208 nerve injuries and 4 202 traumatic amputations were reported, which yields an incidence rate of 13.9 per 100 000 person-yrs for nerve injuries and 5.22 per 100 000 person-yrs for amputations. Although a large amount of injuries occur annually in both categories, most of these injuries are minor injuries, as determined by the corresponding in-patient care time.

Ten nerve injuries fulfilled the stated criteria. Brachial plexus injuries were by far the most care consuming out of these, constituting 5.5 % of the total number of peripheral nerve injuries studied and contributing to 15 % of hospital days spent on peripheral nerve injuries in total. The second most common of the selected nerve injuries was injury of radial nerve at upper arm level (347 cases), followed by injury of peroneal nerve at lower leg level (271 cases). Details on all selected traumatic nerve injuries can be seen in Figure 28a and b.

Complete amputations of body extremities are also relatively rare, since the vast majorities (81 %) of all amputations are amputations of fingers, part of hand or wrist, or toes. These types of injuries are not usually considered for prosthetic solutions and are therefore of minor consequence for this report. Reported injuries, involving complete loss of hand or foot, are in total over the nine years 173 cases and 522 cases respectively. The most common such upper extremity amputation is between the elbow and wrist with 72 cases in total. Correspondingly for lower extremities (disregarding the minor amputations), traumatic amputation between knee and ankle is the most common injury with 215 cases in total. Only five amputation diagnoses fulfilled criteria and all of these concerned amputations of lower limbs. Traumatic amputation at level between knee and ankle stood out as most frequent of these with 215 cases, and these amputations alone also stands for 17 % of the care time spent on treatment of amputations in Sweden. Details on all selected traumatic nerve injuries can be seen in Figure 28 c and d.

In conclusion, the most important target for neural engineering techniques, aimed at improving nerve regeneration after a traumatic injury, should be the brachial plexus injury, since these injuries are both frequent, and requires a large amount of hospital resources. Brachial plexus injuries are however a complex target, and other relevant injuries, more proximal and thereby also easier targets, would be injuries to the radial, peroneal or sciatic nerve, based on data presented in Paper V.

Traumatic amputations between knee and ankle are, although 23 cases per year (Swedish population, 9 million inhabitants) does not constitute a large amount of injuries, relatively frequent compared to other amputations. The same technologies that would apply for such neuroprosthetic legs could presumably also be useful for other traumatic leg amputations.
Figure 28: The selected traumatic nerve injuries constitute 230 injuries annually in Sweden. a) show specific diagnoses and their contribution to these traumatic nerve injuries, b) show the corresponding number of days (out of 2900 days in total) spent in in-patient care unit on the treatment of these injuries.
Figure 28: The selected traumatic amputations constitute 48 injuries annually in Sweden. c) show specific diagnoses and their contribution to these traumatic amputations, d) show the corresponding number of days (out of 1200 days in total) spent in in-patient care unit on the treatment of these injuries.
From the data presented in Paper I, II and IV and also from the literature (Ludwig et al., 2006, Cui and Zhou, 2007, Cui and Martin, 2003, Nyberg et al., 2007, Nyberg et al., 2002), it is obvious that PEDOT electrodes have several properties that are very attractive for the neural interface. First, it can easily be tailored, both mechanically, electrochemically and biochemically, to encourage neural ingrowth and attachment and reduce glial encapsulation. Furthermore, it also provides alternative approaches to the multi-electrode array, either through the woven electronics presented in Paper IV or as electrode material on very flexible substrates like PDMS as shown earlier by Nyberg et al. (2002). In the author’s opinion the use of PEDOT is not fully exploited when silicon substrates are used. Although some additional mechanical buffering can be achieved through growing the polymer in gel scaffolds (Kim et al., 2004), one should take advantage of both the possibility to work with other types of substrates and include organic logic functions.

Although some report PEDOT material with conductivities up to 1000 S/cm (Winter-Jensen et al., 2008), the coated monofilaments discussed here have conductivities an order of magnitude smaller. Better methods for fibre coating would contribute to fibres with higher conductivities, but it is possible that PEDOT despite this will not reach the range required for certain applications, and that hybrid systems employing both metal conductors and polymer conductors will be the best compromise. For the woven electronics such hybrids can easily be implemented using either thin fibres of pure metal, or metallised Kevlar fibres like the ones described by Lawrence et al. (2003). It would also be interesting to look at the possibility to use carbon nanotube yarns, as shown by Zhang et al. (2004), in such hybrid systems. These yarns, produced by spun carbon nanotubes, can be fabricated with diameters in the size range 2-10 µm and with a conductivity of 300 S/cm.

Previous authors have already shown the potential of using thin structures to minimise the foreign body response (Seymour and Kipke, 2007, Stice et al., 2007, Sanders et al., 2000). Paper IV shows that WECT weaves would add to this, through providing a whole new opportunity to build large networks of such fibres with built in addressing logic. The possibility to build electronics in three dimensions is also pointed out as a powerful tool to switch from flat and stiff devices to more complex geometries more similar to biological structures. Thin wires will need an additional component for charge delivery and, although not shown in experimental work here, we believe that such a component, like the one in Figure 27, is straight forward to make. In conclusion, we recognise that there is also a lot of additional work needed, optimising transistors, coatings and circuit design, to truly make this technology useful for the suggested application. It is our belief that the advantages of the woven electrode array would make such efforts worthwhile.

One route to improve stability of already existing silicon multi-electrode arrays would be to use conducting polymer material to improve material/cell interaction. PEDOT decrease interfacial impedance and increase the charge per pulse that can be injected reversibly from a platinum surface. Results, with further positive implications for the use of PEDOT for stimulation, can be found in a study by Sahin and Tie (2007) analysing the most effective waveform to use when stimulating neural tissue, as determined by an electrical equivalent.
model of the mammalian axonal membrane. Their results show that exponentially decaying pulses are more effective to use than rectangular pulses for activation of the axonal membrane. This further supports the use of PEDOT coatings on stimulating electrodes, since the current response to a square wave voltage pulse applied on a PEDOT electrode is, in fact, already an exponentially decaying current (Figure 22).

Paper I also shows that functionalization of the polymer film with biomolecules can easily be done, and in the authors opinion this is also something that should be done, considering it is highly likely that every effort will be needed to rescue neurons and mediate the glial scar formation. One way to do this, retaining the vast part of PEDOT’s attractive electroactive properties, is by using heparin as counter ion in the polymerisation process. The inclusion of heparin in the material is especially interesting considering that several growth factors in turn can bind to heparin (Sakiyama et al 1999, Cole and Glaser, 1986) and hence, the resulting material might be used for the binding of such growth factors. This might be a route to incorporate also growth factors that for some reason can not be used as counter ions in electropolymerisation, and it is also possible that this could be a way to perform controlled release of these substances in the future. Such experiments are however not included in this thesis, although it would be interesting complementary work, to the results presented here, to see if the heparin bound to PEDOT is still available for the binding of other molecules. The Toluidine Blue assay, included in Paper I, shows that at least a fraction of the heparin incorporated in the PEDOT film is available for reaction with the Toluidine Blue, which is an indication of that this could also be the case for heparin-binding peptides. If true, it would open up for a whole set of functionalized PEDOT surfaces, since the diversity of heparin-binding peptides and proteins range from growth factors, cell adhesion molecules, angiogenesis as well as anti-angiogenesis proteins, and even antimicrobial heparin-binding peptides exist (Andersson et al 2004, Chon et al, 2001).

For an implant to be considered safe it is important that possible risks concerning implant related infections have been evaluated. A porous surface might be desirable from the electrochemical and tissue interaction perspective, but can also be a surface with increased risk of bacterial infection and proliferation. It is well known that dead spaces, not readily accessible for blood borne immune cells, are a risk factor for such infections, and can not only be a cause of implant failure but also result in life-threatening conditions for the patient. It is therefore possible that the most relevant biomolecules to incorporate in the material are antimicrobial and not growth factors.

Another interesting development of the PEDOT:heparin material would be to investigate, more thoroughly, how the PEDOT/biomolecule composite can be developed into a hydrogel electrode with improved charge transfer and mechanical properties. Although not included in the experimental work here, it is highly likely that the same method used by Ghosh and Inganäss (1999), making use of PEDOT:PSS dispersion for the gel, would work well also when using heparin as counter ion. An alternative route, that once again completely excludes the use of surfactants, would be to use a new type of alkoxy sulfonate PEDOT called S-PEDOT. S-PEDOT is a water soluble form of PEDOT with anionic sulfonate groups on the side chains. It is expected to be biocompatible, and a preliminary cytotoxicity test, similar to the elution test presented in Paper II, show good survival of SH-SY5Y cells in the presence of S-PEDOT (Figure 29, not included in papers). Although it might improve
the electrochemical properties of the hydrogel electrode, it has already been pointed out by others (Kim and Martin, 2004) that a conducting gel is not a necessity for polymer growth in scaffold structures. This means that when it comes to gels the selection is just as wide as for the counter ions, and the gel constituents can also be chosen for optimal material tissue interaction. A simple route would be to use bivalent ions and the biomolecule used in electropolymerisation to form the scaffold gel on the electrode, or to use the inherent viscoelastic properties of a HA solution. It would also be interesting to investigate the possibility to use gels suggested by other authors to act as neuron attractive coatings (Winter et al., 2007, Sakiyama et al. 1999). This will be included in future work to further optimise electrode biocompatibility. The protocols used for material testing in this report (Paper I-III) can also easily be applied after more material modifications have been made, to identify possible problems concerning electroactivity, stability and biocompatibility early in the process.

One important conclusion of this thesis, with implications for future work, is the stability issues identified in Paper III. Firstly, delamination of films can be the most severe threat to electrode stability if improper substrates are used. Further investigations both considering how film adherence can be improved, and also covering what happens with PEDOT debris after is has delaminated, are important topics to include. Possible negative consequences on surrounding tissue from PEDOT debris and degradation products would be a good complement to the biocompatibility studies included here. Paper III also points out that inclusion of heparin, and presumably also other biomolecules, might negatively influence PEDOT stability. The reason for this should be studied in more detail and once the mechanism has been identified, proper measures can be taken to counteract this effect.

Figure 29: Elution cytotoxicity test of S-PEDOT. Positive and negative controls are the same as for tests described in Paper II.
On the other hand, also the stability of PEDOT:PSS was unsatisfactory, which point to the conclusion that the most relevant topic of continued research on PEDOT neural interfaces would be to address the problem of insufficient stability in biological environment. The intention with introducing PEDOT, to the field of neural electrodes, is to improve stability of the neuron/electrode coupling both through increasing charge delivery capacity, enhancing compatibility with neurons and reduce glial scar formation. If, on the other hand, the favourable properties of PEDOT does not persist for the expected life time of the neuroprosthetic implant, this must be taken into consideration when designing the device. Replacing one stability issue with another is hardly the way to proceed to solve a problem. Either PEDOT stability must either be improved or the consequences of PEDOT degradation must be dealt with accordingly. It is not clear whether temporary improvements during the first month of implantation are sufficient to reduce glial scar formation and rescue neurons long term. If that is the case, a PEDOT coat designed to enhance biocompatibility at an early stage and subsequently be degraded, could be an acceptable solution. If so the remaining function of the implant, after the electroactivity of PEDOT is lost, must still be sufficient for device functionality.

Another possible solution to the problem would be a device implementation which allows refilling of the PEDOT electrode when needed. A publication from the University of Michigan (Richardson-Burns et al, 2007) shows that it is indeed possible to electropolymerise PEDOT in situ in living tissue, and this would not only be a route to refill the implant but is also suggested to be a possible way to infiltrate the glial scar. The authors suggest that in situ electropolymerised PEDOT could be grown through the gliotic tissue and reach out to neurons beyond the scar, i.e. using the scar tissue similarly to the scaffold of a hydrogel. Although refillable electrodes are an extremely interesting approach, there are also many serious concerns with in situ electropolymerisation, especially considering the distribution of the toxic and very reactive monomer in delicate tissue. Another perhaps less daunting path to the same results would be to use a dispersion of already polymerised PEDOT, as in the Baytron-M PEDOT:PSS, or the surfactant free alternative S-PEDOT. Implants with microfluidic channels have already been developed (Retterer et al, 2004) and distribution of the polymer, rather than the monomer, stand greater chance of approval and reduce the risk of insurmountable biocompatibility issues.

One additional area where conducting polymers might be used to improve neuroelectronic implants, not covered explicitly by this thesis, is to control stiffness of implants at different time points. One problem using very flexible substrates is that the forces needed for manipulation of the implant on insertion into tissue places lower constraints on implant stiffness. To cause minimal volume displacement of tissue the implant should be as thin as possible, and to be able to penetrate the tissue the inserted material must therefore be rather rigid. Once inserted, it is on the other hand desirable to have an implant with flexibility in the same range as surrounding tissue. Conducting polymers have previously been used to build a wide selection of electronically controllable micro actuators (Immerstrand et al, 2002, Zainudeen et al, 2008), and it would be interesting to look into their applications for tuneable stiffness properties of implants as well. Through a successful implementation of electronically controllable material stiffness, it might be possible to replace the stiffer silicon arrays with truly flexible polymer arrays in the future.
It is evident that there are many possible ways to deal with the problems of current neuroelectronic implants. Conducting polymers can be part of the solution in more aspects than just as a coating material. Although some problems still exist with the conductivity and stability of these polymers, hybrid metal/polymer implants might be the right way to get the best performance out of the two worlds. Whether that should be done through the woven logic presented here, or as electroactive coatings on planar flexible substrates, remains to be determined. The ideas presented in this thesis can be developed further, and the methods used to characterise material electroactivity, stability and biocompatibility, should be part of this iterative process. It is our hope that the final result of this process in turn could open up the possibility for truly selective long term stable neural interfaces, including all its concomitant applications.
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Composite biomolecule/PEDOT materials for neural electrodes

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Received 21 July 2008; accepted 10 September 2008; published 10 November 2008


Electrodes intended for neural communication must be designed to meet both the electrochemical and biological requirements essential for long term functionality. Metallic electrode materials have been found inadequate to meet these requirements and therefore conducting polymers for neural electrodes have emerged as a field of interest. One clear advantage with polymer electrodes is the possibility to tailor the material to have optimal biomechanical and chemical properties for certain applications. To identify and evaluate new materials for neural communication electrodes, three charged biomolecules, fibrinogen, hyaluronic acid (HA), and heparin are used as counterions in the electrochemical polymerization of poly(3,4-ethylenedioxythiophene) (PEDOT). The resulting material is evaluated electrochemically and the amount of exposed biomolecule on the surface is quantified. PEDOT:biomolecule surfaces are also studied with static contact angle measurements as well as scanning electron microscopy and compared to surfaces of PEDOT electrochemically deposited with surfactant counterion polystyrene sulphonate (PSS). Electrochemical measurements show that PEDOT:heparin and PEDOT:HA, both have the electrochemical properties required for neural electrodes, and PEDOT:heparin also compares well to PEDOT:PSS. PEDOT:fibrinogen is found less suitable as neural electrode material. © 2008 American Vacuum Society.

DOI: 10.1116/1.2998407
Biocompatibility of PEDOT/biomolecular composites intended for neural communication electrodes

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**Abstract**
Electrodes of the conjugated polymer poly(3,4-ethylene dioxythiophene) (PEDOT) have been shown to possess very attractive electrochemical properties for functional electrical stimulation (FES) or recording in the nervous system. Biomolecules already present in nervous tissue, added as counter ions in PEDOT electropolymerisation, could be a route to further improve the biomaterial properties of PEDOT, eliminating the need of surfactant counter ions like docedyl benzene sulphonate (DBS) or polystyrene sulphonate (PSS) in the polymerisation process. Such PEDOT/biomolecular composites using heparin, or hyaluronic acid, have been electrochemically investigated in a previous study and have been shown to retain the attractive electrochemical properties already proven for PEDOT:PSS.

The aim of the present study is to evaluate biocompatibility of these PEDOT/biomolecular composites *in vitro* and also evaluate PEDOT:heparin biocompatibility in cortical tissue *in vivo*. Hereby, we also aim to identify a suitable test protocol, that can be used in future evaluations when further material developments are made.

Material toxicity was first tested on cell lines, both through a standardised agarose overlay assay on L929 fibroblasts, and through elution tests on human neuroblastoma SH-SY5Y cells. Subsequently, a biocompatibility *in vivo* test was performed using PEDOT:heparin coated platinum probes implanted in the cerebral cortex of Sprague-Dawley rats. Tissue was collected at three weeks and six weeks of implantation and evaluated by immunohistochemistry.

No cytotoxic response was seen to any of the PEDOT:biomolecular composites tested here. Furthermore, elution tests were found to be a practical and effective way of screening materials for toxicity and had a clear advantage over the agarose overlay assay, which was difficult to apply on other cell types than fibroblasts. Elution tests would therefore be recommendable as a screening method, at all stages of material development. In the *in vivo* tests, the stiffness of the platinum substrate was a significant problem, and extensive glial scarring was seen in most cases irrespective of implant material. However, quantification of immunological response through distance measurements from implant site to closest neuron, and counting of macrophage densities in proximity to polymer surface, was comparable to those of platinum controls. These results indicate that PEDOT:heparin surfaces were as compatible with cortical tissue as pure platinum controls.

**Keywords:** biocompatibility, conducting polymers, PEDOT, neuroprosthetics
Stability of PEDOT materials intended for implants

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Abstract
This study presents a set of experiments designed to study the stability over time of the conducting polymer poly(3,4-ethylene dioxythiophene) (PEDOT), under simulated physiological conditions. Especially, the influence of switching the counter ion used in electropolymerisation, from surfactant polystyrene sulphonate (PSS) to heparin, was investigated. Electropolymerised PEDOT was exposed to different solutions at 37 °C over a 5-6 weeks study period. Two methods were used to study changes over time, spectroscopy and cyclic voltammetry. Phosphate buffer solution (PBS) and diluted hydrogen peroxide (H$_2$O$_2$) (0.01 M) were used to simulate in vivo environment. Some PEDOT electrodes in PBS were also subject to voltage pulsing to further stress the material.

The vast part of the samples of both types lost both electroactivity and optical absorbance within the study period, when exposed to H$_2$O$_2$. An overall slightly higher stability of PEDOT:PSS compared to PEDOT:heparin could be seen. The time dependence of the decline also differed, with a linear decrease of electroactivity for PEDOT:heparin while for PEDOT:PSS a comparably stable appearance initially, followed by a marked decrease after 8-15 days.

Polymers were relatively stable in PBS throughout the study period, with around 80% of electroactivity remaining after five weeks. Disregarding a slight drop in electroactivity during the first day, voltage pulsing in PBS did not increase degradation (tested over 11 days). Delamination of PEDOT exposed to PBS was however a significant problem, especially for polymer on ITO substrates.

PEDOT is sensitive to oxidising agents, also in the dilute concentrations used here, and counter ion influences the time course of degradation. Even without oxidising agents, some decline in electroactivity can be expected and it is unclear whether this decrease will continue over time, or if the polymer will stabilise. Such stabilisation was however not seen within the five weeks studied here. Delamination of polymer is likely to be a problem on implantation, especially with unwisely chosen substrates, and might be an even more serious threat to long term applications than degradation in biological fluids.
Abstract—New strategies to improve neuron coupling to neuroelectronic implants are needed. In particular, to maintain functional coupling between implant and neurons, foreign body response like encapsulation must be minimized. Apart from modifying materials to mitigate encapsulation it has been shown that with extremely thin structures, encapsulation will be less pronounced. We here utilize wire electrochemical transistors (WECTs) using conducting polymer coated fibers. Monofilaments down to 10 μm can be successfully coated and weaved into complex networks with built in logic functions, so called textile logic. Such systems can control signal patterns at a large number of electrode terminals from a few addressing fibres. Not only is fibre size in the range where less encapsulation is expected but textiles are known to make successful implants because of their soft and flexible mechanical properties. Further, textile fabrication provides versatility and even three dimensional networks are possible. Three possible architectures for neuroelectronic systems are discussed. WECTs are sensitive to dehydration and materials for better durability or improved encapsulation is needed for stable performance in biological environments.

Index Terms—conducting polymers, functional electrical stimulation, textile electronics
Incidence of traumatic peripheral nerve injuries and amputations in Sweden between 1998 and 2006

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Accepted for publication in Neuroepidemiology, November 2008

Abstract

Background: To define the epidemiological pattern of nerve injuries and traumatic amputations in Sweden, 1998-2006, and investigate possible targets for emerging neural engineering and neuroprosthetic technologies.

Methods: The Swedish Hospital Discharge Register was used as basis of information, including data from all public in-patient care, excluding out-patient data. ICD-10 codes were screened for nerve injuries and traumatic amputations of high incidence or in-patient care time. Selected codes, causing factors, age and gender distribution were discussed in detail, and potential targets for tailored solutions were identified.

Results: Incidence rate was determined to 13.9 for nerve injuries and 5.21 for amputations per 100,000 person-yrs. The majority of injuries occurred at wrist and hand level although it could be concluded that these are often minor injuries requiring less than a week of hospitalization. The single most care consuming nerve injury was brachial plexus injury constituting, in average, 68 injuries and 960 hospital days annually. When minor amputations of fingers and toes were disregarded, most frequent site of amputation was between knee and ankle (24 patients / year).

Conclusions: Based on analysis of incidence and care time, we find that brachial plexus injuries and lower leg amputations should be primary targets of these new technologies.

Keywords: Traumatic nerve injury, Traumatic amputations, Epidemiology