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Development of a polymer based neural probe

*How to record intracortical neural activity
while minimizing the tissue response.*

Fredrik Ejserholm



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Doctoral thesis, March 18, 2016

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Title: Development of a polymer based neural probe - <i>How to record intracortical neural activity while minimizing the tissue response.</i>		
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*Only two things are infinite, the universe and human stupidity,
and I'm not sure about the former.*

Albert Einstein

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Cover illustration

Front: SEM image of μ Foil probes used in **Paper III and IV**.
Back: Photo of the author in a teletubbie/clean room suit.

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Abbreviations

- CO₂ - Carbon dioxide
- CVD - Chemical vapor deposition
- DAPI - 4',6-Diamidino-2-phenylindole
- DBN - 1,5-Diazabicyclo[4.3.0]non-5-ene
- DBS - Deep brain stimulation
- DMSO - Dimethyl sulfoxide
- DMTA - Dynamic mechanical thermal analysis
- FDA - US Food and Drug Administration
- GC-MS - Gas chromatography mass spectroscopy
- GFAP - Glial fibrillary acidic protein
- HDPE - High-density polyethylene
- HF - Hydrofluoric acid
- IrO_x - Iridium oxide
- LC-MS - Liquid chromatography mass spectroscopy
- LFP - Local field potential
- MTT - 3-(4, 5-Dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide
- OSTE+ - Off-stoichiometry thiol-enes + epoxy
- PC - Polycarbonate

Abbreviations

- PD - Parkinson's disease
- PEDOT - Poly(3,4-ethylenedioxythiophene)
- PFA - Paraformaldehyde
- PSS - Polystyrene sulfonate
- PT-Black - Platinum black
- PVD - Physical vapor deposition
- RIE - Reactive ion etching
- ROI - Region of interest
- SEM - Scanning electron microscopy
- SNR - Signal to noise ratio
- STN - Sub thalamic nucleus
- T_g - Glass transition temperature
- TiN - Titanium nitride

Preface

The whole plan after high school and a year in the army was to enroll into an engineering program, microelectronics, at the Royal Institute of Technology in Stockholm (KTH). This was, however, changed when my father one day said, "Fredrik, you can not go to school in Stockholm, you should go to a University where there is a strong linkage to the students". He handed me a copy of the magazine *Ny Teknik* where a new engineering program, Nanoscience, was listed in Lund, and said, "There is some kind of new program in Lund where they do small things as well". So without my father, I would probably have moved to Stockholm and never written this book.

I started the nanoscience program in 2004, and I took some time off for some skiing in the spring of 2007. The fall of 2007 and the spring of 2008 I spent working in the student union. I continued my education in the fall of 2008, and then in the spring of 2009 I was enrolled into a course, sensors, that you normally take in your third year, but for me it was in the end of my fourth year. It was an interesting course and in the end of it I asked the teacher, Lars Wallman, if he had a master thesis project that I could do for him. He had one that was about modifying electrodes with a conductive polymer called PEDOT. This thesis was presented and finished in November 2009, and I knew that Lars and my other supervisor on the thesis, Martin Bengtsson, had funding for a Ph.D. student. And after discussing the position with them, I applied and got the position.

The project that I was intended to do as a Ph.D. student was a continuation on my master thesis, but now on neural probes instead of general electrodes. It turned out that the project never really took off, there were so many more interesting projects available. I still kept the project alive by supervising two master theses on the matter.

My Ph.D. project has evolved during the last couple of years. From starting with surface modifications on neural probes, to development of the probe itself, to *in vivo* biocompatibility tests of probes, to using the probe. After a conference that I attended the focus of my Ph.D. project shifted back to development of the probe, but this time towards the material used as the backbone in the probe. The new material was a polymer, OSTE+, which had a tunable flexibility. The project involving OSTE+ forced me into new areas such as

polymer development, characterization of the mechanical properties of polymers, *in vitro* and *in vivo* biocompatibility tests as well as chemical analysis using mass spectroscopy.

It has been a long journey that has taken me almost six years, and basically it's because of my father. And as the final step of this journey, is this book, my thesis. So lean back, grab a cup of tea and just enjoy reading: my thesis.

Acknowledgements

So it's been roughly twelve years since I moved to Lund in order to get my master in Engineering. And I do not know if anyone at that time thought that I still would be here twelve years later, not even me. It has been a long journey and now my time in academia is starting to run out. During these twelve years a lot has happened, it was actually close that my time in Lund ended in 2010 when I had the opportunity to start a Ph.D. at EPFL in Lausanne Switzerland.

The last seven years that I have been part of this department Lars Wallman and Martin Bengtsson has been a big part of my life. They not only supervised my master's thesis but also this, my Ph.D. thesis. I am very grateful and thankful to both Walle and Martin for "taking care" of me during the last seven years. And imagine that it all started because I went skiing for three months.

This last couple of years have not always been a walk in the park, but without my colleges here at the department, my friends and family it would probably not have been possible for me to complete this. So I would like to thank all of you!

Ola, Calle and Linus, thank you for sharing an office with me. Belinda for always being there and for organizing all the different events at the department. Anette for working with me at both Elmät/BME and NRC and for all grateful discussions. Ulrika and Malgo for helping me with everything from my extra salary to all the invoices. Désirée for keeping tabs on all of us Ph.D. students, as well as the "ordinary" students. Mikael for all the help with projects.

I would like to thank Jens Schouenborg for allowing me to work with and learn from NRC. I would like to thank Henrik Jörntell for allowing me to work in his lab and for all the guidance. I would like to thank Per Köhler for all the hours spent working with our electrodes. Marcus for learning me so much and allowing me to give input on the projects. Susanne for all help during surgery. Jonas for learning me tons and for all the time spent in the labs. Palmi for all help with analyzing data and great discussions. Anders for being there and for all the discussions regarding probe designs. Christopher for help with analyzing and general tech talk about probes.

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I would like to thank Alexander Vastesson and Tommy Haraldsson for introducing me to OSTE+ and learning me all there is to know about it. I would like to thank Annika Weiber and Matilda Larsson for all the help with the DCS and DTMA. I would like to thank Jan Åke Jönsson and Margareta Sandahl for learning and showing me how to use the HPLC, LC-MS and GS-MS. I would like to thank John Stegmayr and Stina Oredsson for the collaboration with all the *in vitro* biocompatibility work done on OSTE+. I would like to thank Heui Chang Lee and Kevin Otto for the collaboration with the *in vivo* biocompatibility work of OSTE+.

I would like to thank Börje, Carlos and Ola for all the lunches and the chats that we had regarding research in general, supervisors and just life.

Rick and Henry, thank you for proofreading not only my master thesis, an article but also this, my Ph.D. thesis.

Eva and Tomas, thank you for all the support and help with William through out the last couple of years.

I would like to thank my father, who sadly is not with us anymore, for all the support and good talks that we have had, I only wish there could have been more of them. Thank you mom for being there and always having time. Thank you Calle for being a role model throughout my engineering degree, you received your engineering degree in time and that made me work harder so that I not only received my engineering degree but not also my Ph.D. degree on time.

William, my son, I love you and all the joy you have brought me so far is incredible and I am so proud of being your father! Finally, I would like to thank my wonderful wife, Anna! Without you this would never been done! Jag älskar dig och du kommer alltid att finnas hos mig!

Summary

Intracortical neural probes have in the last couple of years been developed from stiff single probes to stiff multi-electrode neural probes to flexible multi-electrode neural probes. One reason for the change in design is that more than one recording/stimulation electrode, as in the case with a single wire, is needed for more advanced studies. The stiff multi-electrode probes were fabricated using silicon as the base and the stiffness of silicon made the probes unable to follow the micro motions of the brain. This led to the development of polymer based multi-electrode probes that are better to follow the micro motions of the brain. The polymer based neural probes, when comparing to a stiff probe, have less tissue response, reduced encapsulation of the probe by glial cells, increased functional neurons around the probe and a smaller void around the probe in the tissue. It has also been shown that the encapsulation around the neural probe is the largest around the base of the probe, while at the edges the encapsulation is smaller.

When designing a neural probe used for neural recordings, it is of interest to keep the electrical impedance of the interface between the electrode and the tissue as small as possible, since it will generate a signal with a better signal to noise ratio than if the electrical impedance is large. The impedance can be decreased by increasing the size of the electrode, but if the size of the electrode gets too large it might act as a direct connection between two neurons, hence the size should be as small as possible. The solution to this is to modify the electrode surface to become porous/rough, which will keep the geometrical size of the electrode small and at the same time keep the active area of the electrode large.

With all this in mind, my work has mainly been about designing a flexible polymer based intra cortical neural probe for chronic recordings. The probe, designed by me and my colleges, reassembles a flat and flexible Christmas tree and on the tip of each branch there is an electrode. There are 9-13 platinum black modified recording electrodes on the probe. The platinum black modification decreases the electrical impedance of each electrode by roughly one order of magnitude. In one study the probe recorded neural activity from mossy fibers and climbing fibers in the cerebellar molecular layer in rats. In one study it was used to analyze how hyperalgesia can be seen in the somatosensory cortex of free

moving rats. Not only has the overall design of the probe been developed over time, but also the material out of which the probe was constructed. The polymer has been changed first from SU-8 to polyimide, and then to a newly developed polymer called OSTE+. I further developed OSTE+ from its original use in micro fluidic devices. Polyimide has the benefit over SU-8 that it is not as brittle and OSTE+ has the benefit over polyimide and SU-8 that it can be up to 500 times more flexible. Since OSTE+ is a newly developed polymer two biocompatibility studies were done; the first one used *in vitro* MTT assays together with mass spectroscopy for analyzing the biocompatibility and in the second one *in vivo* immunohistochemistry was used for the biocompatibility studies. It was shown that OSTE+ is rendered nontoxic to cells if it is incubated in water for one-week prior to use and that the tissue response of OSTE+ compared to polyimide is similar.

- In **Paper I** - a neural probe constructed in SU-8 with nine gold electrodes was fabricated and tested *in vivo*. Each electrode was modified with platinum black, which lowered the electrical impedance by approximately a factor 10.
- In **Paper II** - it was shown the implantation direction for probes matters to the tissue response, where the flat side of the probe should be in the coronal plane.
- In **Paper III** - SU-8 was abandoned in favor of polyimide as the backbone in the probe for a couple of reasons: SU-8 is brittle compared to polyimide, polyimide is easier to work with and the biocompatibility of SU-8 is still not investigated thoroughly. The change in material also allowed for a new design, where the number of electrodes was increased from 9 to 13 and tested *in vivo*.
- In **Paper IV** - the probe developed in **Paper III** was used for a study to determine if mustard oil could be used as a pain model regarding hyperalgesia in awake free moving animals, where it was possible to see both primary and secondary hyperalgesia as well as primary and secondary allodynia by recording the neural activity when stimulating the animal either with a CO₂ laser or by mechanical stimulation.
- In **Paper V** - the polymer was once again changed to an in-house further developed polymer, OSTE+. OSTE+ has the benefit that the flexibility of the material can be tuned by changing the ratio of the constituents in the mix, which leads to a mixture that at 40 °C is roughly 100 times more flexible than SU-8 or polyimide. A prototype of a neural probe with nine gold electrodes was constructed.
- In **Paper VI** - the biocompatibility of OSTE+ was tested using *in vitro* MTT assays, using polyimide and HDPE as positive controls. The analysis showed that OSTE+ was toxic, but if it was incubated in water for a week before the test it was non-toxic. The samples were further analyzed using mass spectroscopy together with dose response tests for all the constituents, but no connection between the pure constituents and the toxicity was found. Hence, the toxicity has to have come either from impurities in the constituents or from synergetic effects of the different constituents.

-
- In **Paper VII** - the biocompatibility of OSTE+ was further tested but this time *in vivo*. This time probes constructed of different materials were tested *in vivo* for eight weeks. The different materials compared were silicon (Young's modulus, flexibility, of roughly 150 GPa), polyimide (Young's modulus of roughly 3 GPa) and two different blends of OSTE+: one with a Young's modulus of 300 MPa at 37 °C and 6 MPa at 37 °C, which is roughly 500 times more flexible than that of polyimide. The results were that polyimide probes had less tissue response than silicon probes and that the tissue response from OSTE+ were similar to that of polyimide.

Populärvetenskaplig sammanfattning

Hjärnan är en del av kroppen där vi fortfarande inte förstår allt. Den senaste forskningen har gett oss massor med kunskap, men dock saknas en hel del. Ett sätt att få reda på hur de olika delarna av hjärnan fungerar är att förstå hur hjärnceller pratar med varandra och vad de säger. Ett av de bästa sätten för att förstå hur något fungerar är att titta på saken när den arbetar. Nu kan man inte titta in i hjärnan och se hur den fungerar, utan man får lyssna på den istället. De signaler som hjärnceller använder för att prata kan vara av två olika slag. De signaler som skickas mellan två hjärnceller då de pratar med varandra består av olika sorters kemikalier, medan de signaler som skickas inom själva hjärncellen när den pratar med andra hjärnceller är elektrisk. Att mäta vilka och hur mycket kemikalier som en hjärncell släpper ut när den pratar med en annan hjärncell är väldigt svårt, men det är relativt enkelt att mäta de elektriska signalerna. Förenklat kan man säga att man mäter de elektriska signalerna genom att stoppa in två kablar in i hjärnan och sedan mäta spänningen med en voltmeter. På detta sätt lyssnar vi på hjärnceller idag.

Dock är problemet att om man stoppar in något in i kroppen så kommer det att kapslas in på ett eller annat sätt. Inkapslingen gör det svårare att uppfatta de elektriska signalerna då inkapslingen fungerar som en barriär. Hjärnan ligger även omsluten av vätska innanför skallbenet, och när vi rör på oss skumpar hjärnan omkring där inne.

De verktyg eller de implantat som vi forskare använder för att lyssna på och även prata med hjärnceller, är idag något mer avancerade än bara två kablar. Ofta vill man inte bara lyssna på ett ställe i hjärnan, utan på flera olika ställen samtidigt. Detta har gett oss implantat som har flera olika elektroder, punkter, som kan man lyssna med.

Eftersom hjärnan rör på sig inne i skallen och har en konsistens som påminner om rumstempererat smör, så kommer man att få ett stort hål i hjärnvävnaden och en tjock inkapsling, om man stoppar ner ett hårt implantat. Men om man stoppar ner ett mjukt implantat så får man ett mindre hål och även mindre inkapsling. Man kan tänka på det som att om man tar ett paket med rumstempererat smör, sticker ner en smörkniv i smöret och

vickar på smörkniven, så får man ett stort hål. Hade man istället stoppat ner något mjukt som till exempel gladpack och vickat på det, så får man ett mindre hål. Detta har gjort att man har börjat använda och utveckla hjärnimplantat där basen är gjord i mjuk plast istället för hårt kisel som man tidigare använt. På implantatet finns flera elektroder som används för att lyssna på hjärnceller. Man vill även att elektroden ska vara så liten som möjligt, för med en stor elektrod finns en risk att man kortsluter två hjärnceller som då inte fungerar på samma sätt som innan. Nackdelen med att göra elektroden liten är att det elektriska motståndet ökar när storleken minskar. Ett stort motstånd ger en signal med mycket bakgrundsbrus i signalen och det vill man undvika. För att lösa detta har man på olika sätt modifierat elektroden så att man får en skrovlig yta. Den skrovliga ytan gör att man får en stor aktiv yta som får plats på en liten geometrisk yta och man får därmed en liten elektrod som har ett litet elektriskt motstånd. Det gör att man får en stark signal från hjärnimplantatet med lite störningar eller brus.

Jag har under min tid som doktorand arbetat med att bygga hjärnimplantat som har många elektroder, 9-13 stycken och som är byggd i plast så att den bättre kan följa hjärnans rörelser. Elektrodernas ytor har även modifierats med platina som gör att den liknar strukturen av en blomkål. En fördel med den metod vi har använt för att bygga våra implantat är att det är enkelt att ändra hur hjärnimplantatet ser ut och på så sätt kan ändras så att det passar hjärnans olika strukturer. Jag har även vidareutvecklat en ny sorts plast, OSTE+, vars mjukhet kan ändras genom att ändra mängden av de olika ingredienserna som ingår i plasten. Fördelen med denna plast är att den kan göras upp till 500 gånger mjukare än de plaster som används idag. Då detta är en ny plast har vi testat om plasten är giftig för celler. Vi såg att om man låter plasten ligga i vatten en vecka så var den inte giftig för cellerna. Vi har även testat plasten i hjärnor på möss för att se om plasten är giftig i hjärnan. Samtidigt testade vi även om det är någon skillnad på hur mycket inkapsling det blir med den nya plasten jämfört med de plaster som används idag. Det vi såg var att den nya plasten inte påverkar hjärnan mer än de plaster som redan används.

Det hjärnimplantat som jag har tagit fram kan ändras så att det passar olika delar av hjärnan, det är flexibelt och kan bättre följa hjärnans små rörelser och man får ut en stark signal utan så mycket brus när man mäter hjärnaktivitet med det. Jag har även vidareutvecklat en ny sorts plast för användning i hjärnimplantat som är mycket mjukare än vad som finns tillgängligt idag, vilket gör att ett implantat gjort av denna plast borde följa hjärnans rörelser bättre.

List of papers

- I. **A polymer based electrode array for recordings in the cerebellum**
F. Ejserholm, P. Köhler, M. Bengtsson, H. Jorntell, J. Schouenborg, L. Wallman
5th International IEEE/EMBS Conference on Neural Engineering, 376–379, 2011, Cancun.
- II. **Influence of Probe Flexibility and Gelatin Embedding on Neuronal Density and Glial Responses to Brain Implants**
P. Köhler, A. Wolff, F. Ejserholm, L. Wallman, J. Schouenborg, C. E. Linsmeier
PLoS ONE, 10(3), March 2015.
- III. **μ-Foil Polymer Electrode Array for Intracortical Neural Recordings**
F. Ejserholm, P. Köhler, M. Granmo, J. Schouenborg, M. Bengtsson, L. Wallman
IEEE Journal of Translational Engineering in Health and Medicine, 2:1–7, 2014.
- IV. **Altered nociceptive and tactile input to primary somatosensory cortex during an episode of TRPA1 mediated hyperalgesia – implications for assessments of pain in animals**
P. Köhler, P. Thorbergsson, F. Ejserholm, J. Thelin, M. Granmo, M. Bengtsson, L. Wallman, J. Schouenborg
(manuscript)
- V. **A polymer neural probe with tunable flexibility**
F. Ejserholm, A. Vastesson, T. Haraldsson, W. van der Wijngaart, J. Schouenborg, L. Wallman, M. Bengtsson
6th International IEEE/EMBS Conference on Neural Engineering, 691–694, 2013, San Diego.
- VI. **Biocompatibility of a polymer based on Off-Stoichiometry Thiol-Enes + Epoxy (OSTE+) for neural implants**
F. Ejserholm, J. Stegmayr, P. Bauer, F. Johansson, L. Wallman, M. Bengtsson, S. Oredsson
Biomaterials Research, 19(1):1, 2015.

VII. Is the flexibility of neural probes significant for the tissue response?

H. Lee[†], F. Ejserholm[†], S. Currin, J. Gaire, J. Schouenborg, L. Wallman, K. Otto
and M. Bengtsson
(manuscript)

[†] Authors share first authorship

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Introduction

Why would we like to be able to talk to neurons?
How can we talk to neurons?
Can we do it better?

This thesis will try and give answers to these three questions. I hope that you will enjoy this thesis as much as I have writing it.

1.1 Why would we like to be able to talk to neurons?

”Why would we not want to” or ”because it would be cool” are probably the first answers that pop up in my head when thinking about it. But if we would disregard these, the possibility to help people with conditions associated with the the brain and/or our pursuit to learn more about how the brain itself functions would be the biggest driving force for being able to talk to neurons.

Humankind’s ability to help people with conditions associated with the brain have evolved from “only” alleviating symptoms originating from different kinds of trauma to the skull or the ability to exorcise demons that caused seizures (epilepsy), during the Mesolithic and Egyptian age¹⁻⁴ to the ability in modern times to help people with conditions ranging from tumors, neurodegenerative diseases such as Parkinson’s disease (PD) to allowing deaf people to hear again, blind people to see again and paralyzed people use their paralyzed limbs again.

Today we are able to relieve patients of symptoms from several conditions using a neural probe with which we can talk and listen to neurons. We can even get neurons to start talking (excite them) or stop talking (inhibit them). E.g. in PD where the loss of dopaminergic cells in the basal ganglia leads to a cascade where neurons in the sub thalamic nucleus (STN) are no longer inhibited, here a neural probe can be used to inhibit the STN to relieve the symptoms from the PD.⁵ The ability to listen to neurons allows the paralyzed to move a cursor on a computer or even control a robot arm just by thinking.^{6,7} There is also the possibility both to listen and talk to neurons, e.g. in patients with epilepsy

where neurons have an excessive and abnormal activity. Before the seizures that occur in some patients there are several neurons that have an abnormal activity. By listening for the abnormal activity using a listening neural probe it is possible to inhibit the neurons that are causing the seizure with a stimulating neural probe.⁸

1.2 How can we talk to neurons?

Neurons communicate with each other using neurotransmitters (certain specific chemicals) but within neurons the communication is based on electricity. Since it is easier to measure electricity than concentrations of different chemicals, electricity is used both to listen and talk to neurons. This allows us to listen to neurons by measuring the electrical potential around the neuron as well as talk to neurons by stimulating the neuron with electricity (at 30–60 Hz to excite the neuron and above 100 Hz to inhibit the neuron).⁵ The tools that we use to record neural activity or stimulate neurons can either be non invasive or invasive; this thesis will be limited to invasive intra cortical neural probes for chronic use. Invasive neural probes have the ability to have a higher signal to noise ratio (SNR), can be closer to the neurons and the resolution is better compared to non invasive interface.⁹

1.3 Can we do it better?

This is the 100 dollar question, and the rest of this thesis will try and answer that question. First we have to see how neural probes affect the brain. After this we can try and make the probes invisible for the brain itself, increase the longevity and effect of the probes.

1.4 How do intracortical neural probes affect the brain tissue?

An intracortical neural probe requires that the surgeon first remove the skin around the area of interest on the skull, then the bone has to be opened up, followed by removal / penetration of the meninges to allow the neural probe to penetrate the neural tissue. The insertion of the neural probe will induce an acute inflammatory response that involves the activation and migration of microglia towards the neural probe.¹⁰ After the acute reaction has faded a chronic tissue response is triggered where a glial scar will encapsulate the neural probe. The glial scar will then act as an insulator, which will insulate the probe from the tissue and therefore lower the SNR.¹¹

How can we minimize the encapsulation?

Let's start with an early Michigan type neural probe^{12–14} that basically is a flat sword-like probe made out of a rigid material, in this case silicon, that we tether the probe to the skull. There are several different ways to minimize the tissue response by allowing

the neural probe to follow the micro motions of the brain. Since the brain is floating in cerebrospinal fluid, when we move our head we will set our brain in motion in aspect to the skull, which will make a tethered probe move in aspect to the neural tissue, which will increase the inflammatory response and the encapsulation. To minimize this response, one could untether the neural probe from the skull, make the neural probe in a flexible material to allow it to follow the micro motions or design the neural probe so that it will anchor itself to the neural tissue. This thesis will focus on constructing the probe in a flexible material as well as allowing the neural probe to anchor itself to the neural tissue by its design.

1.5 Aims

The aim of this thesis is to develop intracortical neural probes for chronic recordings of neural activity. We decided that we were to focus on a flexible neural probe constructed in a polymer that should allow the probe to follow the micro motions of the neural tissue. We have then divided this aim into two greater parts:

- Develop a probe in an already known polymer with a design where the probe itself is anchored into the neural tissue and where the recording electrodes are moved away from the bulk material.
- Develop a new polymer to be used in neural probes that is even more flexible than what is currently available.

1.6 Outline

Chapters 2 and 3 will provide an overview about how neurons communicate and how cells and neural tissue respond to foreign objects. **Chapter 4** is about the neural probe; what designs are available and out of which material they can be made, how the tissue response from the probes can be minimized and how the active part of the probe (the electrode) functions. **Chapter 5** deals with the neural probe my colleagues and I developed, the μ Foil, and how and why it has evolved during the last couple of years.

1.7 Historical overview

It was 1664 when Jan Swammerdam showed a Danish botanist (Olaf Borch) the first evidence of how nerves function. He used a scalpel with which he stimulated the nerve endings of a frog, making its muscles contract. In 1667 Swammerdam published his doctoral thesis, in which he induced movements in a dog's diaphragm by tactile stimulation to the nerve endings.^{15–18} The next breakthrough was in 1791 when Luigi Galvani discovered that he could make a frog's muscles contract when stimulating the nerves using electricity. His work also included how the contraction of a muscle and the intensity of the electrical stimulation relate to each other (that there is a limit after which an increase

1. Introduction

in the stimulation intensity will not increase the contraction of the muscle) as well as that there is a refractory period after a stimulation before another stimulation will contract the muscle.^{19–21}

Giovanni Aldini, Galvani's nephew and assistant, continued Galvani's experiments by moving on to warm blooded animals. In the winter of 1802 he performed spectacular experiments on decapitated prisoners in Bologna, making their muscles contract when stimulating them with electricity. In fall of 1802 Aldini was able to treat a 27-year-old farmer who was suffering with melancholy madness by repeated electrical stimulation between the farmer's hand and head; some might say that Aldini performed the first electroconvulsive therapy session. Aldini and Carlo Mondini worked on stimulating the human brain and they were able to get the facial muscles to contract when stimulating the corpus callosum. They also stimulated the cortex of one of the hemispheres inducing muscle contractions on the other side of the head.^{22,23} The first and correctly interpreted neural recording from a nerve was done by Carlo Matteucci in 1838. He was able to measure the current flowing through a frog's leg when it was electrically stimulated.²⁰ In 1850 Herman von Helmholtz was able to record the speed of a propagating nervous signal (later known as the action potential).

Gustav Fritsch and Eduard Hitzig continued Aldini's research by stimulating different areas of a dog's brain, resulting in the movement of the limbs of the dog showing that the brain was connected throughout the limbs through nerves.²⁴ In 1874 Roberts Bartholow reported the first findings when stimulating the brain in an awake human. He also reported that when he stimulated the left side of the brain the leg and arm on the right side moved.²⁵ In 1902 Fedor Krause had performed 142 experiments on humans where he stimulated the cortex. He was able to create the first map of the motor cortex from these findings. He was also able to prove that the work Fritsch and Hitzig did on dogs also was true on humans.²⁶ From the 1920s Walter Rudolf Hess did the first in-depth studies on the functional organization of the brain; he used wires to stimulate deep structures in freely moving cats. He was awarded the Nobel prize in 1949 together with Egas Moniz.^{27,28}

The way to cure/relieve patients of their conditions for movement disorders such as PD during this time was done by lesions in the brain. But without proper support and knowledge the mortality was high. In 1947 Ernest Spiegel and Henry Wycis developed both the stereotactic frame and the first stereotactic atlas over the human brain. Both allowed neurosurgeons to reach deep structures without damaging the surrounding tissue in a precise, more safe and reproducible manner.

Roughly during the same time as Spiegel and Wycis developed the stereotactic frame, Alan Lloyd Hodgkin and Andrew Huxley described²⁹ in detail how the action potential was built up and conducted in the nerves. Hodgkin and Huxley received the Nobel price in medicine in 1963 for their work.

It was not until 1987 that Alim Louis Benabid showed that it is possible to use electrical stimulation to inhibit neural structures using a stimulation frequency above 100 Hz. Before this the main use for electrical stimulation was to treat pain in patients. Benabid showed that it was possible to get the same effect that the neurosurgeon gets with lesions

by using using electrical stimulation for movement disorders.⁵ And it paved the road for Deep Brain Stimulation (DBS) probes that we today use for movements disorders.

The research today has come far from the first experiments that Swammerdam performed on frogs; today there is research being done on everything from movements disorders, epilepsy, Alzheimer's, mental disorders, restoring sight, restoring hearing and even allowing paraplegic control of robot limbs.

2

Cell Communication

The nerve cell, a neuron, communicates with another neuron using different neurotransmitter chemicals and the communication within the neuron is electrical. This chapter will give the background needed to understand how neurons communicate, how the signal is created and how to listen to it.

2.1 The neuron

The neuron, **Fig. 2.1**, is a cell that generally is composed of: one or several dendrites, a single cell body (soma), a single axon and one or several axon terminals. The function of the dendrite is to receive neurotransmitters released from other neurons. The soma contains the nucleus, and the surface of the soma is covered by voltage gated ion channels, and it is here that the incoming signal from the dendrites is amplified and sent further across the axon. The axon is the cable between the soma and the axon terminal, it is covered by voltage gated ion channels whose sole function is to propagate the signal (the action potential) from the soma to the axon terminal. Around the axon there is a myelin sheath which isolates the voltage gated ion channels on the axon, leaving only small openings called nodes of Ranvier. The isolation and the openings increase the speed of the signal, which is necessary when transporting a signal long distances. At the end of the soma is the axon terminal, where the neuron releases vesicles containing neurotransmitters.^{29,30}

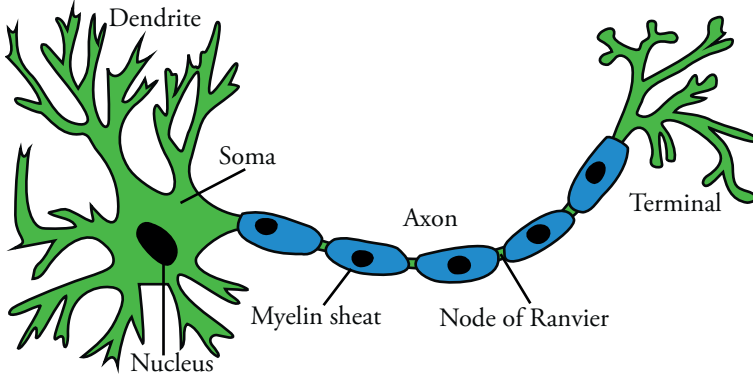


Figure 2.1: A schematic model of a neuron. Modified image originally created by Quasar Jarosz at Wikimedia Commons.

2.2 The membrane potential

The membrane potential or the electrical potential between the inside and outside of a neuron was first described by Hodgkin and Huxley in 1952,²⁹ and the membrane potential, V_{in} , is given by:

$$V_m = V_{in} - V_{out} \quad (2.1)$$

where V_{in} and V_{out} are the potential inside and outside the neuron.

The membrane potential depends on the difference in concentration of ions on either side of the membrane. The most significant ions are sodium (Na^+), potassium (K^+) and chlorine (Cl^-); for each ion there is an energy based pump that works to keep a stable concentration of each ion inside the neuron. For the stable state it is possible to calculate the equilibrium potential for each ion using Nernst's equation:

$$E_X = \frac{RT}{zF} = \ln \frac{[X]_o}{[X]_i} \quad (2.2)$$

where E_X is the Equilibrium potential for ion X, R is the gas constant, T is the temperature in Kelvin, z is the valence of the ion, F is the Faraday constant and $[X]_{o/i}$ is the concentration of the ion inside and outside the neuron. When the neuron is in its stable state the potential (resting potential) over the membrane can be calculated using Goldman's equation:

$$E_m = \frac{RT}{F} = \ln \frac{P_{K^+}[K^+]_o + P_{Na^+}[Na^+]_o + P_{Cl^-}[Cl^-]_i}{P_{K^+}[K^+]_i + P_{Na^+}[Na^+]_i + P_{Cl^-}[Cl^-]_o} \quad (2.3)$$

where P_X is the membrane permeability to ion X (in cm/s). When the neuron is at rest the net ion flux across the membrane is at equilibrium and the membrane potential is usually between -50 and +90 mV.²⁹⁻³¹

2.3 The action potential

The action potential is the signal sent across the neuron from the soma, across the axon, to the axon terminal.

In the neuron the signal starts in the dendrites where a neurotransmitter released by another neuron binds to it and activates Na^+ channels, allowing Na^+ to flow into the cell. This will depolarize the membrane across the soma, and if enough Na^+ channels are activated to get the membrane potential above its threshold this will trigger an action potential. The depolarization of the membrane activates most of the voltage gated Na^+ channels and some K^+ channels, allowing even more Na^+ to flow in and some K^+ to flow out of the cell. The Na^+ channels will inactivate when the action potential reaches its top, while the K^+ channels will remain active. Then the membrane potential will depolarize due to the outflux of K^+ , and it will eventually even overshoot, depolarizing the membrane potential below the resting potential. This will inactivate the K^+ channels, and the membrane will eventually reach its resting potential. The whole process can be seen in **Fig. 2.2**. It takes some time (a few ms) before the Na^+ and K^+ channels change their state from inactivated into their resting state; this time is referred to as the refractory period and it was first discovered in 1791 by Luigi Galvani¹⁹ (see **chapter 1**). The refractory period can be divided into two phases; the absolute phase and the relative phase. During the absolute phase the neuron can not create another action potential, but during the relative phase the neuron can still create an action potential but the threshold is increased.²⁹⁻³¹

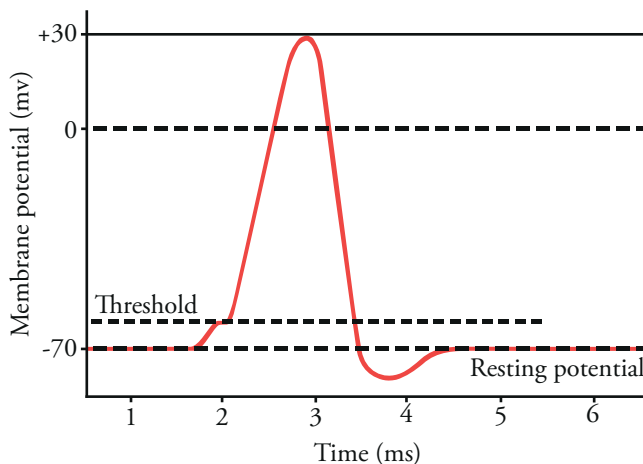


Figure 2.2: The action potential of a typical neuron.

2.4 Neural recordings

There are two types of neural recordings: extracellular and intracellular, where the intracellular recordings are recordings from inside the neuron and extracellular are recordings from the outside of the neuron. This thesis will only focus on extracellular recordings since it allows the researcher to record neural activity without damaging the neuron itself.

The extracellular intracortical neural recordings are made using a neural probe with one or several electrodes. The electrodes are generally made out of some kind of metal with some kind of insulator around the metal, leaving only a part of the metal exposed to the tissue, this is the electrode. The recording made using the electrode consists of several different components, whereas in this thesis I will focus on two of them. The first component is the spike and if the electrode is close enough to a neuron (less than $50\ \mu\text{m}$)³² it is possible to record the action potential propagating through the neuron. Hence, the spike is a direct representation of the action potential. The second component is the local field potential (LFP), which consists of the all the excitatory and inhibitory dendritic potentials from neurons located up to $350\ \mu\text{m}$ from the electrode.^{30,31,33}

During a recording session it is possible to record spikes from several neurons (called a multi unit, whereas a single unit is the action potential of a single neuron) as well as the LFP for each electrode on the neural probe. This makes it hard to interpret and analyze the data, but modern computer software allows us to split the signal so that it is possible to “listen” to one of the neurons at a time as well as seeing the whole picture.

3

Cell and tissue response of foreign materials

Whether a foreign object is biocompatible or not is one of the most important questions when working with medical devices. First, there is no foreign object that is truly biocompatible, i.e., the body will always react to a foreign object. Throughout this thesis, when discussing biocompatibility, the tissue response is discussed. So if an object or material is called biocompatible, this means that there is no significant change in tissue response caused by the implanted material.

As stated in the introduction of this thesis (**chapter 1**), there will be a tissue response when inserting a neural probe into the tissue. The difficulty, however, lies in seeing what the response to this will be. This chapter will go more in depth and explain two different methods to analyze the tissue response.

When a foreign object (e.g., a neural probe) is inserted into brain tissue it might abjoin the extracellular matrix, capillaries, blood vessels and disrupt the blood brain barrier. The probe might even penetrate the cell membrane of cells that were in the way of the probe during the implantation. The mechanical trauma done to the blood vessels will activate platelets, clotting factors and the complement system, which together aid in the rebuilding process of the damaged tissue. And the trauma done to the tissue will activate microglia that will migrate towards the probe, where they will release cytokines and free radicals around the wound. If the probe is removed at this stage the wound will eventually heal and what is left is a small glial scar. However, if the probe is left in the tissue there will be a chronic inflammation from the tissue where both activated microglia/macrophages and reactive astrocytes will be present around the probe. The astrocytes will start to encapsulate the object in order to shield of the tissue from the probe. The encapsulation will not only push away neurons from the probe but also insulate the probe, and both these effect will make the probe less effective over time and lower the SNR of the electrodes of the probe.^{11,34-37}

3. Cell and tissue response of foreign materials

Two different methods for analyzing the response that a foreign object will have on the tissue are discussed in this thesis: *in vivo* immunohistochemistry (tissue response to a foreign object) and *in vitro* cytotoxicity tests. In **Paper VI**, *in vitro* cytotoxicity tests are used, while in **Paper II and VII** *in vivo* immunohistochemistry is used.

3.1 *In vitro* cytotoxicity – MTT assay

Cytotoxicity assays are used everyday for testing if a substance is harmful to cells. The finding can then either be used as a way to find out if a substance is toxic in order to avoid it but it can also be used to find a substance that is toxic to certain cells, e.g., cancer cells, allowing it to be used in therapy.

There are several different ways to perform cytotoxicity tests and in **Paper VI** MTT assays were used for the evaluation. The MTT assay works when MTT (3-(4, 5-Dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide), a tetrazole, is reduced to formazan by NAD(P)H-dependent enzymes (oxidoreductases and dehydrogenases) of metabolically active cells.^{38,39} The formazan can then be dissolved, and by measuring the concentration of the dissolved formazan in the solution using a spectrophotometer, and comparing it to a control curve, the number of viable cells can be calculated.

When using the MTT assay to investigate the toxicity of a substance, the substance is directly dissolved into cell medium. The type of cells that are going to be used for the tests are then kept in the substance / medium mix for a predetermined amount of time (72 hours were used in **Paper VI**) at 37 °C. For polymers or other insoluble materials, it is necessary to make an extract of the material. In **Paper VI**, the extracts were created by placing pieces of the different polymers into vials with water, the vials were kept at 37 °C for 72 hours. The extract was then mixed into cell medium and then cells were kept in the extract medium mix for 72 hours at 37 °C. After 72 hours, MTT was added to the cells. The cells were incubated with the MTT at 37 °C for one hour, after which the medium was removed and the cells were dissolved in dimethyl sulfoxide (DMSO). The concentration of formazan was measured and the number of viable cells was calculated.

3.2 *In vivo* – immunohistochemistry

When it comes to testing if a material will affect the tissue, *in vitro* tests are simply not enough to get the whole picture. Hence *in vivo* tests will fill in some of the blanks from the *in vitro* tests.

The *in vivo* tests done in **Paper II and VII** utilized *in vivo* immunohistochemistry where neural probes are implanted for X weeks in **Paper VII**) weeks, after which the tissue is analyzed. The technique makes use of antibodies that attach to specific target antigens, e.g., macrophages and cell nuclei. The target can then be visualized by using a secondary antibody with a fluorescent tag attached to it. The secondary antibody being designed so

that it only binds to the first antibody. Then, by using a fluorescent microscope it is possible to see where the antibodies are attached, so that the different cells can be visualized. Two sets of antibodies are used in order to increase the specificity of the detection.^{40,41}

In **Paper II** the probes were implanted for a total of six weeks before the animals were sacrificed, while in **Paper VII** the probes were implanted for eight weeks. For **Paper II** the probes were removed after the animals were sacrificed, while in **Paper VII** the probes (except the silicon probes) were left in the tissue. After the animals were sacrificed all tissues were fixated (to preserve the tissue from degradation) by a transcardially administered perfusion with paraformaldehyde (PFA). After fixation the brains of the animals were removed from the head and perfused again in PFA. Prior to freezing the brains, they had to be cryopreserved, which was done by submerging them into a sucrose solution overnight. The frozen brains were then sliced using a cryostat into thin (30–100 μm) slices and then stained with antibodies. Probes made in SU-8 are brittle and there is a risk that the probes shatter during slicing, hence the SU-8 probes were removed from the tissue in **Paper II**. The stained slices were then photographed using a fluorescent microscope with filters that are specific to the different wavelengths the fluorescent tags on the secondary antibodies express.

The antibodies used for staining in **Paper II** were DAPI, CD68/ED1, GFAP and NeuN. DAPI (4',6-Diamidino-2-phenylindole) binds well to DNA, making it possible to see the nucleus of the cell.⁴² CD68/ED1 is the name of a protein that is found in activated microglia, monocytes and macrophages, and the antibody binds to it. GFAP (Glial fibrillary acidic protein) is a protein expressed in activated astrocytes.⁴³ NeuN is a protein that is expressed in the nuclei of most neurons.⁴⁴ For **Paper VII** CD68/ED1, GFAP and NeuN antibodies were used to stain the slices.

The stains allow us to see how many cells are around the probe, if there are any activated microglia in the area, how big the glial scar is and also if there are still any neurons around the neural probe. If all the neurons have been moved too far away (greater than 350 μm), the neural probe will not be able to pick up action potentials from neurons.

4

The neural probe

When it comes to the different design elements of the neural probe, there are several things to consider. Not only the design of the probe itself, but also what materials to use in the probe and how the tissue response from the probe can be minimized. The active part of the probe, the electrode, is another area that has to be considered, especially when designing the electrode. A badly performing electrode renders the probe useless.

There are several different designs of neural probes with one or more electrodes: the wire probe, the Michigan probe and the Utah array, see **Fig. 4.1**. And each of the different designs has its advantages and disadvantages.

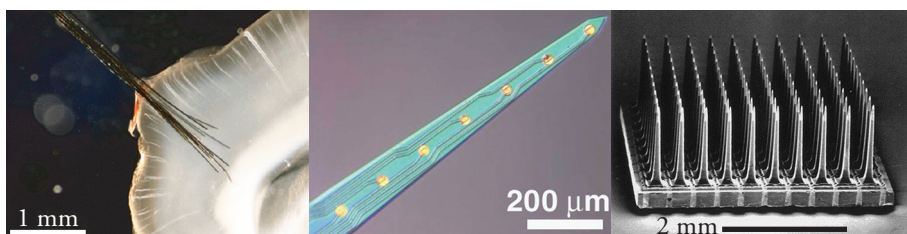


Figure 4.1: Left – 32 7.5 μm tungsten wires bundled together, reproduced from Lind *et al.*⁴⁵ Middle – A Michigan type neural probe reproduced from Abidian *et al.*⁴⁶ Right – An Utah array with 10x10 electrodes, reproduced from Normann *et al.*⁴⁷ All images are reproduced with permission of their respective publishers.

4.1 Wire probe

The wire probe is the most basic probe since it can be created using two insulated wires, one for grounding the animal and one to record the neural activity. For wire probes, the standard is to use either platinum, tungsten or stainless steel.^{48,49} There are however, some

4. The neural probe

more exotic materials used such as platinum-iridium.⁵⁰ The length of the wires depends on which structure in the brain is the target for the probe. The number of wires in the final probe depends on what the study is focusing on, e.g., for mapping areas of the brain a single wire is used, but for recording neural activity in an area several wires are required. The simplicity of wire probes makes it possible for any lab to produce them by itself since it is just an insulated wire and a contact. As such, the fabrication is time consuming and hard, but making small volumes is cheap.

For acute experiments either simple insulated wires or a glass probe, a wire where glass is used as the insulation, is used. The glass/wire probes are fabricated by drawing glass over a sharpened wire. In **Fig. 4.2** we can see a sharpened 75 μm tungsten wire probe that is insulated using glass and a probe consisting of 32 tungsten wires where each wire has a diameter of 7.5 μm together with a 3 μm thick polyimide insulation layer outside the wire.⁴⁵

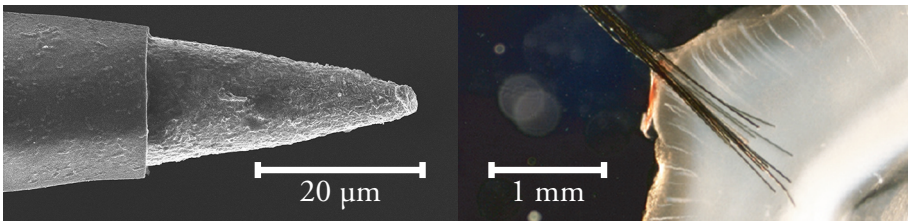


Figure 4.2: Left - A 75 μm sharpened tungsten wire with a glass insulation. Right - 32 7.5 μm tungsten wires bundled together, reproduced from Lind *et al.*,⁴⁵ reproduced with permission from the publisher.

4.2 Michigan probe

The Michigan probe is a flat sword like probe with several individual electrodes generally centered on the flat side, seen in **Fig. 4.3**. Originally the Michigan probe was made out of silicon, but today it is possible to make them out of polymer, which should allow the probe to follow the micro motions better. The size of the Michigan probe depends on how many electrodes the probe has. More electrodes will increase the width of the probe, whereas the thickness varies between 10-50 μm , depending only on material type and user design. The electrodes are generally round, but square versions are available as well, with an area between 200–3000 μm^2 .^{46,49,51–54}

It is possible to make smaller electrodes; it all depends on how good the manufacturer is at making them. Fabrication of Michigan probes requires access to a clean room, which makes it expensive for small volumes, while for larger volumes it is cheap and fast compared to wire probes. Another advantage to the wire probes is that since all the electrodes are at a specific location on the probe, and if a stereotactic frame is used, then the position of

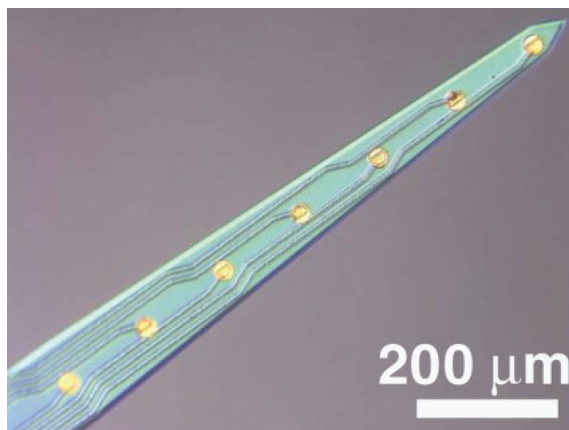


Figure 4.3: A Michigan type neural probe reproduced from Abidian *et al.*⁴⁶ with permission from the publisher.

all the electrodes are known. Compared to the wire probe, especially when using a small diameter, the wires can spread out and the location is not known.⁴⁵

For Michigan probes made out of silicon a thin silicon wafer is used, metal for both the leads and the electrodes are structured using UV-lithography and the typical choice of metal is either gold or platinum. Then some kind of insulation for the leads as well as defining the electrodes is required. This is normally a photoresist that is spin coated onto the wafer, and then structured either using Reactive Ion Etching (RIE) or UV-lithography, and since a metal acts as a stop for the RIE, it is easy to define the electrodes using this method. The probes are then removed from the wafer by etching of the silicon wafer. Finally, the probes are connected either to a circuit board or a contact using either a wire bonder, conductive glue or solder.

For Michigan probes made entirely out of polymer a silicon wafer is used as well, see **Fig. 4.4** for a schematic image of the fabrication. The easiest way to start is to add a release layer onto the silicon wafer by adding a metal: titanium, chromium or nickel are some of the metals used today. Then there are two routes either using RIE to define the probe or UV-lithography. The RIE method is simpler but requires both a RIE machine and a UV-lithography machine. For this method the entire wafer is spin coated with photoresist, then the metal (not the same kind of metal used for the sacrificial layer) for the leads and the electrode is structured using UV-lithography. The insulation layer is created by spin coating a photoresist on the entire wafer. Then the probe as well as the electrodes are defined using UV-lithography and RIE, by removing the unwanted photoresist around the probe and on the electrode sites. For the method where only UV-lithography is used, first the bottom layer of the probe is created by spin coating and structuring a photoresist. Then the metal is structured using UV-lithography, and finally the insulator is defined by spin coating a second layer of photoresist that is structured using UV-lithography. When the probes are defined and ready the sacrificial layer is removed either using electrolysis or

4. The neural probe

by simply etching the metal away. The probes are then connected either to a circuit board or a contact using either a wire bonder, conductive glue or solder.

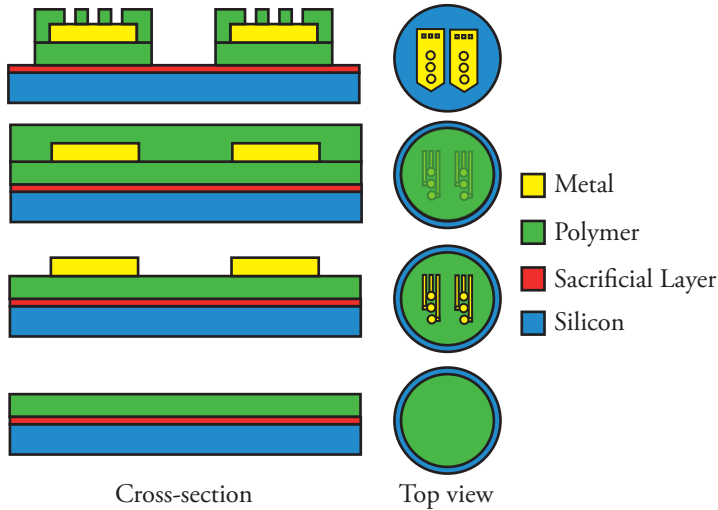


Figure 4.4: A schematic image showing the fabrication steps of a polymer based Michigan neural probe.

Since a clean room and expensive machines are required to make a Michigan probe it is very costly to produce low volumes of the probes. On the other hand since a single wafer can contain up to a hundred or so probes, large volumes are cheap and fast to do. Another benefit for this technique is that it allows the probes to be tailor made for the architecture that is of interest for a specific project.

4.3 Utah array

The Utah array is one of the few probes that is approved by the US Food and Drug Administration (FDA) and it has the resemblance of a spike mat. The Utah array is made out of silicon and has a square, 4x4 mm, base with 100 protruding spikes, see **Fig. 4.5**.⁴⁷ Each spike is an electrode and the size of the electrode depends on how much of the spikes are insulated. The length of the spikes can be altered so that the array fits the selected architecture. An advantage of the Utah array compared to the wire probe is that the location of each electrode is known. Since a clean room and expensive machines are required to make the Utah array they are the most costliest and their fabrication is the most complicated when compared to the wire probe and the Michigan probe.

The fabrication, seen in **Fig. 4.6**, of the Utah array requires a clean room and it starts with a thick p-doped silicon slab, the thickness of the slab representing the total thickness of the

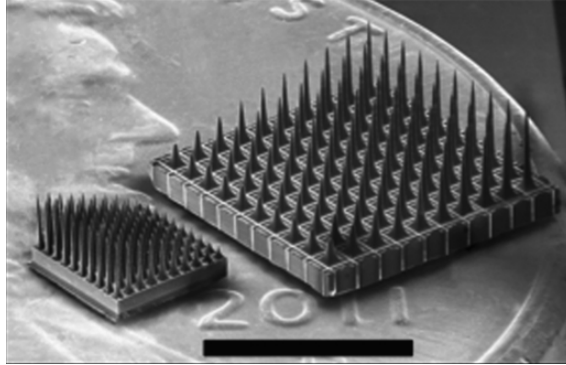


Figure 4.5: Two different versions of the Utah array, the scale bar is 25 μm . The image is reused from Wark *et al.*⁵⁵ with permission from the publisher.

array. On one side of the slab a dicing saw is used to make 300 μm thick groves that are spaced 400 μm from each other. This is done so that one side of the silicon slab resembles a checkerboard, then the groves is filled with glass. The glass is allowed to cool and is then tempered in an oven so that it is hard. Aluminum is then evaporated onto the slab and patterned using UV-lithography so that each square of silicone is covered in aluminum.

Then the slab is turned upside-down where the dicing saw once again is used, but this time it will cut away the silicon leaving an array of 10x10 square spikes. What is left now is a 100 individual silicon spikes that are separated and mounted to a glass slab. Since square blunt spikes are not wanted, the entire array is put into a bath containing hydrofluoric acid (HF) and nitric acid, which will etch away the rough edges and leave a sharp tip on the spikes. A layer of either titanium or tungsten is sputtered onto the spikes, followed by a thin layer of platinum. The spikes are then isolated using polyimide and then the tips are deinsulated using a solvent. On the back of the array each aluminum square is contacted to a connector using a wire bonder. Each slab of silicon has a yield of nine silicon arrays.⁵⁵⁻⁵⁸

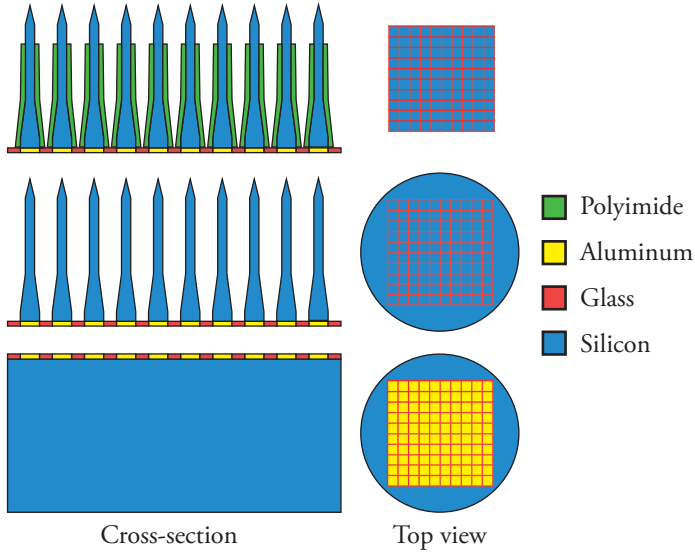


Figure 4.6: A schematic image showing the fabrication steps of a Utah array.

4.4 Different materials used in the Michigan type neural probes

There are two different material types used in a Michigan type probe: silicon or some kind of polymer. The benefit of using silicon is that it is easy to work with since it can be diced, etched, doped and mechanically polished. Silicon is a stiff material, making it easy to insert silicon based probe. The downside is that the probe will not follow the micro motions of the brain, hence there will be a quite large encapsulation.^{11,59} This is also the reason for the development of Michigan type probes manufactured in different kinds of flexible polymers, and this chapter will discuss three different kinds: polyimide, SU-8 and parylene C, that all are roughly 50-100 times more flexible than silicon, see **Table 5.1** for a comparison.⁶⁰⁻⁶⁶ Regarding the biocompatibility of the different polymers, parylene C and polyimide are seen as biocompatible, where parylene C is approved by the FDA, while SU-8 shows good biocompatibility but there seem to be some concerns regarding it still.⁶⁶⁻⁷⁰

Polyimide and SU-8 are both polymers that allow them to be directly structured using UV-lithography. It is also possible to structure them using RIE. When comparing polyimide and SU-8, the largest difference between them is that SU-8 is more brittle, it has an elongation break point of 6.5% compared to 73% of polyimide, and polyimide is slightly easier to work with in the clean room since it doesn't require a post exposure bake, i.e., a heat treatment after being exposed to UV-light, it just have to rest for half an hour before development. We have also noticed during fabrication that there is some shrinkage when using SU-8 that is not seen in polyimide. Since parylene C can not be structured using

UV-lithography directly, it is added to a surface using a physical vapor deposition (PVD) system, and requires access to a RIE in order to be used. Otherwise, it is a material that is even more durable than polyimide (it has an elongation break point of over 200%). There is, however, one downside with using thin parylene C layers as the insulation layer for probes: it has been proven that it leaks current at low frequencies, which could be cumbersome for LFP recordings.^{60-63,66}

4.5 Decreasing the tissue response from neural probes

The acute tissue response is tricky to minimize because the probe has to be inserted into the tissue, and logic tells us that a big and sharp probe should have a larger chance of abutting blood vessels, the extracellular matrix and capillaries as well as damaging cells compared to a smaller and blunt probe. And a single small wire probe should render less tissue response than a large Michigan type probe, but then only a single recording electrode is implanted, which is generally not wanted.^{34,37}

Regarding the chronic tissue response there are several different routes to go. Let's start with the material used to construct the probe itself.

As stated earlier in this thesis, the design of the probe affects the glial encapsulation in such a way that for a Michigan type probe the encapsulation is bigger at the middle of the probe and smaller at the edges. So making the probe thinner overall or placing the electrodes on the edges should allow for a better SNR over time.^{65,71,72}

Probes can be constructed out of either stiff materials or flexible materials, and the reason that the research community currently is moving towards flexible materials is that it should allow the probe to follow the micro motions of the tissue, reducing the tissue response as well as allowing for a stable recording over time.^{73,74} And as discussed before, polymers like polyimide, SU-8 and parylene C are roughly 50-100 times more flexible than silicon, see **Table 5.1**. The downside with using flexible probes is that it is hard to implant them without using some kind of stiffener; either by embedding the probe^{74,75} as done in **Paper II, IV and VII** or by using a stiffener and temporary "gluing" the probe to the stiffener with a degradable "glue" as done in **Paper III**.⁷⁶⁻⁷⁸ As shown in **Paper II** and in the work done by Lind *et al.*,⁴⁵ embedding the probe in gelatin could actually lower the tissue response compared to a probe without gelatin. The orientation of the probe is also of importance for the tissue response as seen in **Paper II**, since most micro motions of the brain will be in the coronal plane. A flexible probe inserted with the flat side in the sagittal plane will act as a stiff probe in the coronal plane and *vice versa*. Hence, a Michigan type probe should be inserted with the flat side in the coronal plane in order to utilize the flexibility to the maximum. It has been shown that a probe with a structured surface have a have less tissue response, than a smooth surface. This is probably because the structured sides will allow for a better adhesion between the tissue and the probe.^{79,80} Anchoring the electrodes in the tissue will allow for a better SNR of the probes over time, since the electrodes will stay in place in respect to the tissue.^{71,81,82}

Another way to decrease the chronic tissue response is the possibility to use bioactive molecules,^{11,46,83–86} mainly peptides, that either reduce the inflammation or increases the adhesion of the tissue to the probe. One way is to incorporate cell adhesion peptides when coating the electrodes of the probe with conductive polymers. The peptides and the polymer will then create an integrated network that allows neurons to adhere better to the electrodes. There is also the possibility of incorporating biodegradable electrospun fibers⁸⁷ that incorporate anti-inflammatory drugs that can be released over time.

The final way to minimize the tissue response is that the probe can either be tethered to the skull or be un-tethered, where the probe is *de facto* floating in the tissue. A study done by Thelin *et al.*⁸⁸ showed that the tissue response from an un-tethered probe is smaller than for a tethered probe. This is probably because that a tethered probe will irritate the tissue at the interface during the micro motions.⁸⁹

4.6 The electrode

The most important part of a neural probe is the electrode, since without a functioning electrode the probe itself is useless. This part of the thesis will start by discussing how the interface between the electrode and the tissue around the electrode. It will also discuss how to modify the surface of the electrodes in order to get a better signal in the end.

There are two mechanisms for transferring charge over the interface between the electrode and the tissue (acting as the electrolyte).^{90–92} The first can be modeled as a capacitor due to that when an electrode is placed in contact with an electrolyte, there will be a redistribution of the different ions in the electrolyte, seen in **Fig. 4.7**. When charged ions in the electrolyte combine with the electrode surface, they create a plane of charge on the surface of the electrode with an opposite charge to that of the electrode. There will also be a gradient of charge over a small distance from the surface of the electrode, until finally the electrolyte will be homogenous again. The double layer created at the surface of the electrode is called the Helmholtz plane. The second mechanism is faradic and it can be modeled as a resistor. This is due to a direct transfer of electrons from the electrode into the ions in the electrolyte due to electrochemical reactions at the surface. The resulting circuit of the resistor and the capacitor can be seen in **Fig. 4.7** and the total impedance from both the resistor and the capacitor depends on the frequency of the signal, i.e. for a low frequency the capacitor will act as a broken lead and the signal will go through the resistor instead.

For a large SNR of the electrode the impedance should be as small as possible. It should however be noted, that the impedance of the interface between the electrode and the tissue will be greater than the impedance within the tissue itself or in the electrode / lead. Hence, it should be possible to use a lesser conducting materials, e.g. conductive polymers, for the leads in the probe. Since a large impedance at the interface will decrease the SNR, decreasing the impedance is desirable and one way of doing this is to increase the size of the electrode. The downside with a large electrode is that it might shunt neurons, and in the worst case short-circuit them. The ideal electrode has an impedance of 0 and a size

that is as small as possible (as close to a point as possible). But, since it is impossible to have both, the only way is to try and increase the active area of the electrode while keeping the geometrical area small. And this can be done by modifying the surface of the electrode by either by removing material so that the surface becomes rough or by adding a material to create a rough surface.

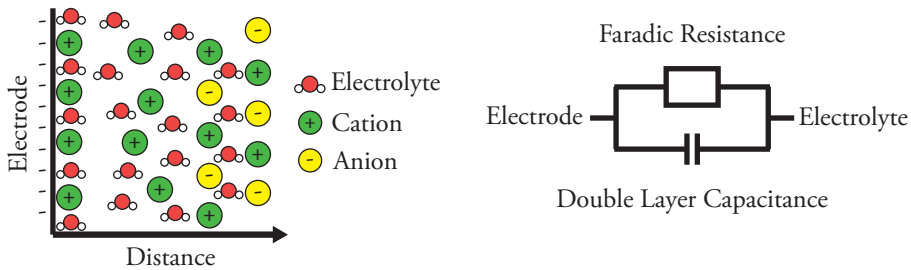


Figure 4.7: Left – Showing a gradient of charge and the Helmholtz plane when an electrode is submerged into an electrolyte. Right – The combined circuit diagram when an electrode is submerged into an electrolyte.

4.7 Different materials used in the Michigan type neural probes

For a stimulating electrode the transfer of charge can either be faradic, capacitive or pseudocapacitive.^{12,90–95} In the faradic mechanism electrons are transferred across the electrode/electrolyte interface and species confined at the surface will be oxidized/reduced. On the other hand, in the capacitive mechanism the charge will induce the transfer of ions in the electrolyte. For the pseudocapacitive mechanism, faradic reactions are confined to a monolayer at the surface of the electrode, which then will induce a transfer of ions in the electrolyte. For stimulating electrodes, a capacitive charge transfer mechanism is preferable, since no chemical species are created or consumed during the stimulation.

Surface modifications to the electrode

There are several different surface modifications used on electrodes today that aim at decreasing the electrical impedance as well as increasing the amount of charge that the electrode can deliver into the tissue before there will be irreversible effects in the tissue or on the electrode. One way to increase the total amount of charge that can be delivered is by increasing the size of the electrode, but as with recording electrodes, there might be a problem with shunting neurons as well as affecting too many neurons.

The different surface modifications this chapter will discuss are platinum black (rough platinum, Pt-Black), titanium nitrate (a conductive ceramic, TiN), Iridium oxide (IrOx)

and poly(3,4-ethylenedioxythiophene) (a conductive polymer, PEDOT). All these different surface modifications work by increasing the active area of the electrode. IrOx and PEDOT have gone beyond the ability to increase the active area; they also have the ability to increase the amount of charge that can be delivered before there will be irreversible effects in the tissue.

Pt-Black

Pt-Black is a surface modification that is electroplated onto the surface, resulting in a rough/porous surface that resembles a cauliflower, as seen in **Fig. 4.8**. The cauliflower indicates that the active surface area of the electrode is increased and the impedance of the electrode is reduced.

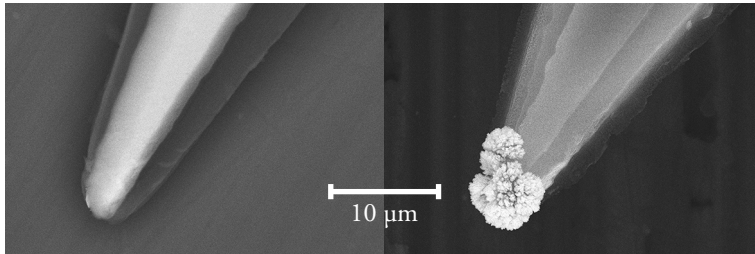


Figure 4.8: SEM images of an electrode on the μ Foil probe before and after platinization with Pt-Black. Modified from **Paper III** with the permission of the publisher.

A Pt-Black modified electrode is pseudocapacitive and the increased surface area makes it possible to use it as a coating on stimulating electrodes.^{91,92,94,96,97} The downside with platinum black is that the protocol requires lead acetate in the electrolyte, which itself is toxic, but there have not been any reported issues with the coating. The biggest concern with the coating is that it might not adhere well to the surface of the electrode. We have however, seen in our work, **Paper I and Paper III**, that since we have a high SNR, enough of the platinum still remains on the electrode. And since such small amounts of platinum are added, it should not affect the tissue substantially. If the coating is applied in an ultrasonic bath during the platinization process, the parts of the coating that have poor adhesion will come loose and the final coating will have a better adhesion. In **Paper I** we saw that Pt-Black decreased the impedance on round electrodes, with a 100 μm in diameter by a factor 10 (at 1 kHz), and in **Paper III** we were able to decrease the impedance on 10 μm^2 electrodes by almost a factor 100 (at 1 kHz), see **Fig. 4.9**.

TiN

TiN is a surface modification that is added either using PVD or a chemical vapor deposition system (CVD), resulting in a porous surface with a large active area compared to the geometrical area.^{90,91,94,98,99} TiN is a ceramic that is seen as biocompatible and since it can be deposited using PVD or CVD the result is an even layer of TiN over all the

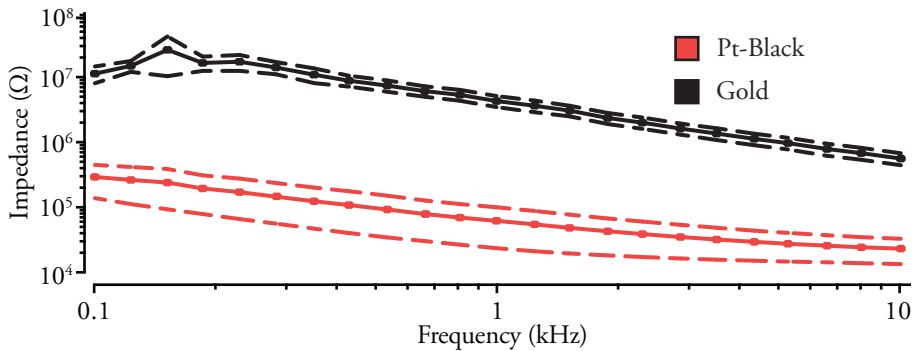


Figure 4.9: A graph showing the impedance for a bare gold electrode and a Pt-Black modified electrode. Modified from **Paper III** with the permission of the publisher. The solid line represents the average and the dashed line represents the standard deviation.

electrodes. The conductivity of TiN is around 23 kS/cm, compared to 4 GS/cm for gold. But since the impedance at the interface is higher than resistivity of TiN, the conductivity is good enough.

IrOX

IrOx is a coating that has the benefit of not only increasing the active area but it also allows for more charge to be injected into the tissue. This is because it is a redox material that can subsequently be oxidized and reduced.^{91,98,100,101} This allows the electrodes to be used for recording but also for stimulation. IrOx has to be activated, i.e. repeatedly oxidized and reduced, before it is used, which is done by pulsing the electrode with a dc voltage. The activation allows a larger amount of charge to be injected into the tissue since it changes the mechanism used to transfer charge into the tissue from a pseudocapacitive to a faradic mechanism. This allows the electrode to transfer charge directly into the tissue.

PEDOT

PEDOT^{51,98,100,102,103} is a conductive polymer that is electropolymerized onto the electrodes and is normally polymerized together with polystyrene sulfonate (PSS) to increase the conductivity of the final coating. As with the IrOx coating, PEDOT has to be activated before being used for stimulation and it uses faradic mechanisms for the charge transfer. Even though PEDOT:PSS has a quite low conductivity of 300 S/cm compared to

4 GS/cm for gold, it is still high enough so that it does not affect the performance of the electrode. PEDOT is considered to be biocompatible, though there is a notion in the research community that the adhesion of PEDOT to the electrode is not the best. During the polymerization of the coating it is also possible to incorporate carbon nanotubes that will increase the active area even further.¹⁰⁴

4.8 The ultimate probe for chronic intracortical recordings

The ultimate Michigan type probe should allow it to follow the micro motions of the tissue, it should adhere well to the tissue, the electrodes on the probe should have a low impedance and the probe should reduce the inflammation around itself. And from the discussion in this chapter it should then meet the following requirements:

- The probe should be constructed put of a very flexible material, allowing it to follow the micro motions of the tissue.⁷³
- The probe should be structured so that it will be anchored into the tissue, allowing for stable recordings over time.^{71,79–82}
- It would be beneficial of the design of the probe easily could be changed, so that the probe can be used for different brain structures.
- The probe should be coated with some kind of biodegradable polymer that contains anti-inflammatory drugs.⁸⁷
- The electrodes should be placed as far away as possible from the bulk material where the encapsulation will be the thinnest, this will increase the SNR.^{11,46,83–86}
- The electrodes of the probe should be modified so that they both incorporates adhesion promoting bioactive molecules as well as a larger active area related to the geometrical area in order to decrease the impedance of the electrode.^{11,46,83–86}
- And finally the probe should be left untethered, which whould allow it to follow the micro motions of the tissue better.^{88,89}

5

μ Foil

The μ Foil is a probe that we have developed during several years and it can be viewed as a further development of the Michigan type probe. This chapter will discuss why we have designed the probe to look as it does, how it has evolved during the last couple of years, how it is fabricated, what kind of surface modifications we have used on the electrode and in the end of the chapter a newly developed polymer used in the probe will be discussed.

Instead of a flat sword like Michigan probe, the μ Foil resembles a flat spearhead or a flat Christmas tree depending on how you look at it, see **Fig. 5.1**. And there are two reasons for the spearhead/Christmas tree design:

- One hypothesis is that the protruding barbs will anchor the probe in the tissue. This together with a flexible probe should allow it to better follow the micro motions of the brain, hence reducing the encapsulation of the probe. The anchoring could also allow the probe to be stable in the tissue, which should allow for stable recordings over long time with a large SNR.^{71,81,82}
- Seymour *et al.*⁷¹ and Skousen *et al.*⁷² have shown that a small probe, when compared to a wide probe, yields a higher neuronal density, lower reactivity of microglia, less activated macrophages and a reduced amount of deposited extracellular protein. Lee *et al.*⁶⁵ showed that electrodes placed at the edge instead of in the bulk of the Michigan type probe have a better recording capability. This gave us the hypothesis that a probe with protruding barbs should have less encapsulation at the tips of the protrusions than at the base and a better recording capability if the electrodes were placed at the tips.

The two hypothesis together should make a probe that has less encapsulation around the base of the probe but also even less encapsulation around the recording sites, which will increase the SNR and the longevity of the probe.

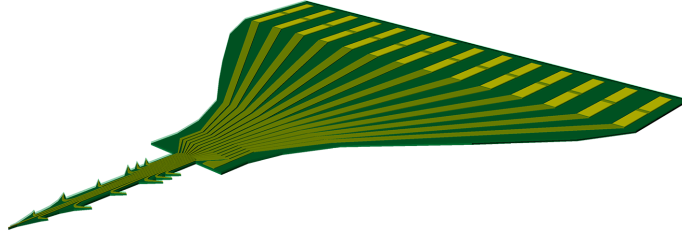


Figure 5.1: A schematic image of the μ Foil probe used in **Paper III and IV**.

5.1 Development

The μ Foil has evolved during the last couple of years starting with a probe consisting of three electrodes with SU-8 as the insulation material. The first change was to increase the electrodes to nine, together with a surface coating on the electrodes consisting of Pt-Black, this is the probe used in **Paper I**. For **Paper III and IV** the material of the base and the insulator was changed from SU-8 to durimide (a type of polyimide), the number of electrodes were changed to 13 and a reference electrode was added. In **Fig. 5.1** a schematic image of the probe used in **Paper III and IV** can be seen, and in **Fig. 5.2** a scanning electron microscope (SEM) image of the same probe. In **Paper V** we changed the material for the base and insulator to a newly developed polymer, OSTE+, and we changed the number of electrodes back to nine. We have not been able to test the OSTE+ version of the μ Foil *in vivo* yet, but we were able to fabricate one as a proof of concept (**Paper V**).

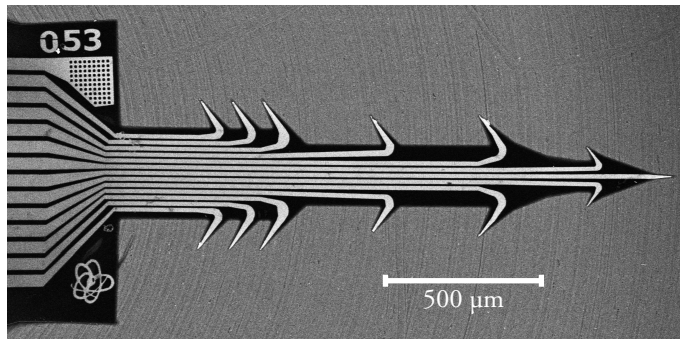


Figure 5.2: A SEM of the μ Foil probe used in **Paper III and IV**. Modified from **Paper III** with the permission of the publisher.

5.2 Fabrication

The fabrication of the μ Foil probe reassembles the fabrication of a polymer based Michigan probe and is visualized in **Fig. 5.3**. It starts with a silicon wafer with a thin sacrificial layer,

onto which the first polymer later is spin coated and structured using UV-lithography. The next step is to add and structure the leads and the electrodes, which is done by evaporation of metals that is structured using UV-lithography. After this the top polymer layer is spin coated and structured using UV-lithography. The final step is to remove the sacrificial layer, hence releasing the probe from the silicon wafer.

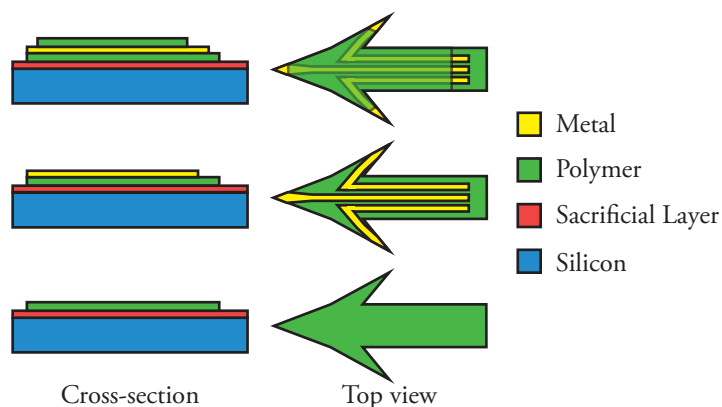


Figure 5.3: A schematic image showing the fabrication steps of the uFoil probe. Modified from **Paper III** with the permission of the publisher.

Since there was a change in materials for bulk of the μ Foil, the fabrication had to change: The sacrificial layer was changed from titanium in **Paper I** to chromium in **Paper III and IV**. The benefit of using chrome instead of titanium as the sacrificial layer is that it does not require the use of HF. There is generally a problem with adhesion between polymers and metals. In order to promote adhesion an adhesion layer consisting of titanium had to be used in **Paper III and IV**. It should be noted that in **Paper I**, no adhesion layer was used and there were some problems with adhesion between the SU-8 and gold leads. On the other hand, in **Paper V** no adhesion layer was used, since gold will bind covalently to OSTE+. The use of an adhesion layer changed the method for patterning the leads from using a positive photoresist as an etching mask, used to etch the metal not of interest, and the use of a lift off technique where a negative photoresist is added before the metal, and then dissolved, lifting away the metal above the negative resist, see **Fig. 5.4** for a schematic figure explaining the different methods. The benefit of the lift off technique is that both the adhesion layer and the lead/electrode can be patterned at the same time.

When the probe finally is released from the wafer by etching away the sacrificial layer, it is attached to a circuit board using solder (**Paper I, III and V**) or an anisotropic conductive film (**Paper IV**). The connection to the circuit board is then isolated using either an epoxy (**Paper I, III and V**) or polyimide (**Paper IV**). For the exact fabrication protocol see **Paper I and III-V**.

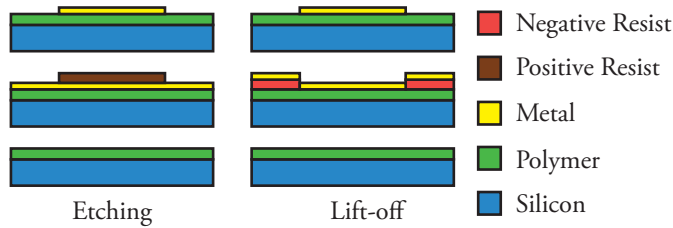


Figure 5.4: A schematic image explaining two different methods for structuring metals.

5.3 Modifications of the electrode

During the development of the μ Foil there has been a development of different modifications to the electrode. In 2009 a master thesis was done (by me) describing the feasibility of using PEDOT:PSS as the coating material for the electrode. However, there were some problems with adhesion of PEDOT, so it was decided to switch to Pt-Black. In **Fig. 4.8** it is possible to see an uncoated and a coated electrode. As seen in **Paper I, III and V**, Pt-Black was used to increase the active area of the electrode. In **Paper I** we saw that the impedance of an electrode with a diameter of 100 μm was decreased by a factor 10 (at 1 kHz), and in **Paper III** we were able to decrease the impedance of 10 μm^2 electrodes by almost a factor 100 (at 1 kHz), which can be seen in **Fig. 4.9**.

5.4 OSTE+

OSTE+ (Off-Stoichiometry Thiol-Enes + Epoxy) is a polymer that was developed at KTH in Stockholm Sweden¹⁰⁵ to be used in microfluidic devices instead of PDMS. OSTE+ is a material that can be spin-coated and structured using UV-lithography and it has a flexibility that depends on the temperature. The reason for developing OSTE+ to be used in neural probes is that even though polyimide, SU-8 and parylene C is more flexible than silicon, there is still a big difference compared to brain tissue (0.5-1 kPa),¹⁰⁶ see **Table 5.1**, while OSTE+, as seen in **Paper V and VII**, has a flexibility that is closer to that of the brain tissue.

OSTE+ consists of three different types of monomers (thiol, allyl and epoxy) that are bonded together using two different initiators. The idea is to create a cross-linked polymer

Table 5.1: Showing the different values of Young's modulus, tensile elongation before the polymer breaks and the glass transition temperature of the polymer.^{60-64,66,107} *depends on the ratio between the constituents.

Material	Young's modulus	Tensile elongation	T _g
Silicon	130-180 GPa	-	-
SU-8	2.0 GPa	6.5 %	210 °C
Polyimide	2.9 GPa	73 %	>350 °C
Parylene C	2.75 GPa	Upp to 200 %	180 °C
OSTE+	4 MPa–2 GPa*	-	0-60 °C*
Neural Tissue	0.5-1 kPa	-	-

where the thiol monomer will be used as the starting base. The polymerization is a two-step polymerization where the first step is a fast UV-activated radical polymerization and the second step is a thermal anionic polymerization. In the first polymerization some of the functional groups on the thiol monomers will bond with all the allyl monomers, and in the second polymerization the unreacted thiol functional groups will bond to the epoxy monomers. The structure of the cross-linked polymer depends on how many functional groups each monomer have. E.g. in **Fig. 5.5** the thiol has three functional groups, the epoxy have two functional groups and the allyl is a blend of two different monomers where one has two functional groups and the other has three functional groups. In the actual mix the number of functional groups might be different and the spider-web will have a different structure.

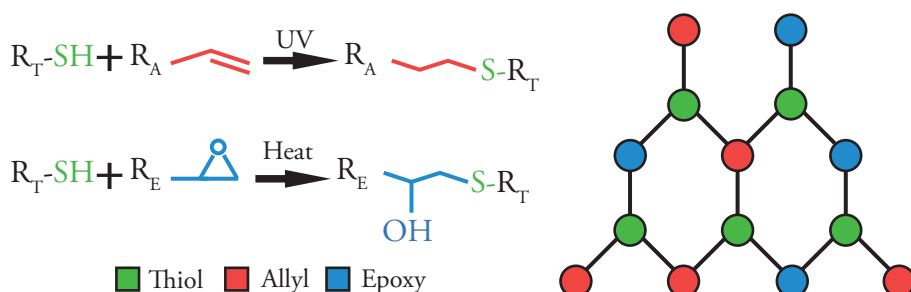


Figure 5.5: Left - A schematic figure explaining how OSTE+ polymerized. Right - The structure of OSTE+ after polymerization when the allyl and epoxy have two functional groups and the thiol has three functional groups. I.e. each blue circle (epoxy) is connected to two green circles (thiol), each red circle (allyl) is connected to either two or three green circles and each green circle is connected to three other circles.

Fabrication of OSTE+

OSTE+ can be structured either by molding or by UV-lithography. In **Paper V** both UV-lithography and molding were used, in **Paper VI** only molding was used and in **Paper VII**

both techniques were used. For an exact statement of the fabrication, please see **Paper V-VII**.

For **Paper V-VII** the OSTE+ was mixed in the same way: all the constituents were weighed and mixed together and then the mixture was degased to remove bubbles. The thiol used was a thiol with three functional groups (tris[2-(3-mercaptopropionyloxy)ethyl] isocyanurate). Two different allyls were used, one with three functional groups (trimethylolpropane diallyl ether) and one with two functional groups (2,4,6-triallyloxy-1,3,5-triazine). For the epoxy (D.E.N 431) there was a mixture of different monomers with different functional groups, the mean value of the functional groups were 1.6. In the first polymerization a UV activated initiator (Lucirin[®] TPO-L) was used, and in the second polymerization DBN (1,5-diazabicyclo[4.3.0]non-5-ene) was used as the initiator. In **Paper V**, acetone was added to the mixture so that it would be easier to pour it into the molds.

In **Paper V** two structures of OSTE+ were fabricated: rectangular OSTE+ bars for mechanical testing and a neural probe prototype, seen in **Fig. 5.6**. For the OSTE+ bars the OSTE+ mixture was poured into a PDMS mold and then the mold with the OSTE+ mixture were covered by a polycarbonate (PC) film. Everything was exposed to UV-light using a mask aligner. After the exposure the PC film was removed and the molds were put into an oven over night, before demolding the bars. The probe consisted of two layers of OSTE+ with gold leads sandwiched between the two different layers. The first layer of OSTE+ was the base and the second layer was used to define the electrode sites and to insulate the leads.

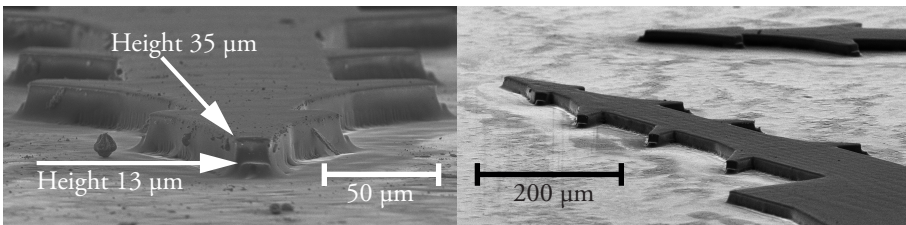


Figure 5.6: SEM images of the OSTE+ based neural probe. Modified from **Paper V** with the permission of the publisher.

The fabrication of the probes started with a silicon wafer with sacrificial layer made out of titanium. The first layer of OSTE+ was spin coated onto the wafer and then covered by a thin PVC cling film before exposure to UV-light using a mask aligner. The wafer was put into butyl acetate to soften up the PVC film, which allowed it to be removed with a pair of tweezers, as well as to remove the unexposed areas of OSTE+. The wafer was then blown dry using nitrogen gas. Gold was evaporated onto the surface and structured using gold etchant and UV-lithography. For OSTE+ there is no need for an adhesion layer between the gold and the polymer. This is because that there might be some unreacted functional groups of thiol left, which will create a strong bond between the gold and OSTE+.¹⁰⁸ A second layer of OSTE+ was structured in the same way as the first layer but with a different

UV-lithography mask. The probe was finalized by submerging the wafer in HF (1:5) for a couple of hours, followed by a thorough washing step in water.

In **Paper VI** bars of OSTE+ were made in the same way as in **Paper V**, using a PDMS mold. Half the bars produced were submerged in water for seven days (OSTE+_{H₂O}), in order to remove eventual leaked constituents.

In **Paper VII** bars of OSTE+ were made in the same way as **Paper V**, and single OSTE+ layered dummy probes were fabricated in almost the same way as in **Paper V**. The difference was that only one layer of OSTE+ was spin coated and structured before the probes were released. All probes were then submerged into water for six days.

Properties of OSTE+

Since OSTE+ is a new material, the different properties of the material had to be investigated. In **Paper V** the mechanical properties of OSTE+ were investigated using Differential Scanning Calorimetry (DSC) as well as Dynamic Mechanical Thermal Analysis (DMTA) and in **Paper VII** DMTA together with water uptake tests were performed. One of the most interesting properties of OSTE+ is that its flexibility can be controlled by changing the ratio of the constituents, allowing a change in the glass transition temperature (T_g), which is seen in **Papers V and VII**. In **Fig. 5.7** the Young's modulus of the different versions of OSTE+ used in **Papers V-VII** can be seen.

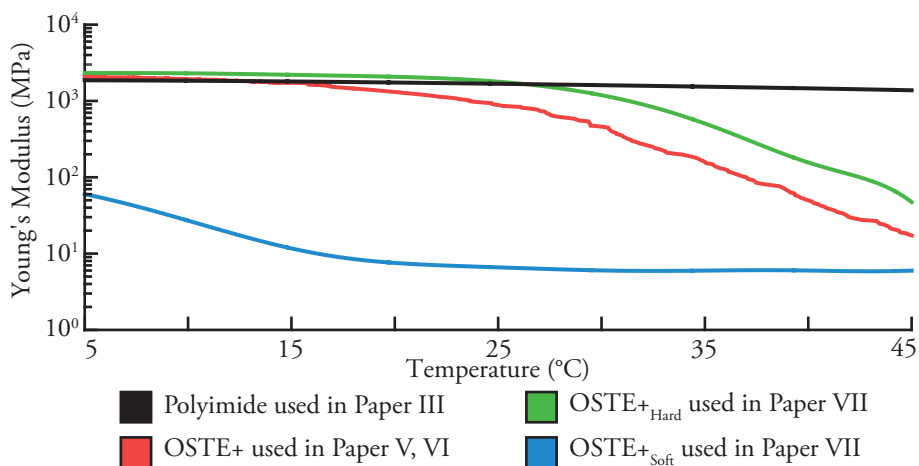


Figure 5.7: A graph of Young's modulus vs temperature of polyimide and the different blends of OSTE+ used in **Paper V, VI and VII**.

As stated before the T_g of OSTE+ depends on the ratio of the different constituents used in the fabrication, but a change in constituents will also change the Young's modulus of OSTE+. As seen in **Papers V and VII**, depending on the temperature and the ratio of the

different constituents used in OSTE+, the Young's modulus of OSTE+ can be between 4 MPa and 2 GPa, which makes OSTE+ up to 500 times more flexible than SU-8, polyimide and parylene C and up to 30,000 times more flexible than silicon, see **Table 5.1** for a comparison of the different materials.

Paper V showed that it is possible to create a neural probe using OSTE+ as the bulk material, and the Young's modulus of the mixture used was 1.9 GPa at 10 °C and 30 MPa at 40 °C. It was also shown that it was possible to cool the probe using a peltier element, which stiffened the probe enough so that it was possible to implant it into agar at 40 °C.

In **Paper VII** two different blends of OSTE+ were used, OSTE+_{Hard} and OSTE+_{Soft}, where the difference between the blends was the ratio of the constituents. The reason for using two different blends was to have two versions of OSTE+; one as hard as possible (300 MPa at 37 °C) and the other as soft as possible (6 MPa at 37 °C), while keeping the constituents the same.

6

Concluding remarks

When it comes to the future of intra cortical neural probes one of the most important areas is to have stable recordings over time and the smallest possible tissue response from the probes. In order to get stable recording over time, a low electrical impedance at the interface between the electrode and the tissue is of interest but also the encapsulation of the electrodes should be as small as possible.

I have shown throughout this thesis that it is possible to lower the electrical impedance by at least one order of magnitude by electroplating the active area of the probe by platinum black (**Paper I, III and IV**). I have also developed a new polymer to be used in neural probes, OSTE+, and we have shown that it is as biocompatible as polyimide (**Paper V–VII**). In **Paper VII** we fabricated and tested OSTE+ that was roughly 500 times more flexible than polyimide *in vivo* together with probes constructed in polyimide and silicon, we did however not see any big significant difference between the two polymer based probes, whereas the silicon based probe had larger tissue response compared to the polymer based probes. This have opened up if probes really have to be more flexible then what they are today. It might be that the tissue response from the probes now come from the fact that there is an object implanted into the tissue.

When it comes to OSTE+, it has some properties that make it suitable to be used in intra cortical neural probes, but since the the fabrication is more complex it is hard to justify the extra work. It should be possible to accomplish the same effect on the tissue response that intra cortical probes constructed in OSTE+ have by using conventional materials such as polyimide and parylene C instead. Since the final flexibility of a probe is based on both the material and the design of the probe, a thinner probe should affect the tissue response in the same way as a more flexible material.

Even though OSTE+ might not be suitable to be used in intra cortical neural probes, it could still be used in probes that nowadays use PDMS. OSTE+ has a couple of properties that makes it more suitable then PDMS: it can be structured in an easy way using UV-lithography, it is not as brittle as PDMS and it can easily be modified with bioactive

6. Concluding remarks

molecules since it contains thiols. The thiols could also enhance the adhesion of metals to the polymer.

When it comes to how the best intracortical neural probe for chronic use should be constructed I suppose that most points have already been written in the “The ultimate probe for chronic intracortical recordings” section in the “The neural probe” chapter (**Chapter 4.8**), but in case you skipped that part (and I don’t blame you)... I believe that intracortical probes in the future should be designed with several active sites on one probe, be flexible so that they can follow the movements of the brain, the design of the probe should be easy to change so that the probe can be fabricated to function with different brain structures, incorporate active biomolecules so that they can reduce both the acute and the chronic inflammation, be as thin as possible to increase the flexibility, should not be tethered to the skull, should be embedded in some kind of soft material during implantation, the active sites should be kept small and the active site should be modified so that the electrical impedance is as low as possible.

Finally, I hope that you have enjoyed reading my thesis as much I did writing it. And thank you for reading it!

Best regards,
Fredrik Ejserholm

7

Summary of the Papers

7.1 Paper I

A polymer based electrode array for recordings in the cerebellum

In this paper we show a 8-12 μm thick polymer probe with nine electrodes, see **Fig. 7.1** for a photograph of the probe. The base of the probe was constructed in SU-8, the electrodes and the leads were made in gold with an adhesion layer of chromium. Since the electrodes were small ($10 \mu\text{m}^2$), causing a high impedance, the electrodes were coated using Pt-Black. We tested the effect the platinum black had on the impedance on $100 \mu\text{m}$ test electrodes, and we saw that the impedance at 1 kHz were lowered by one order of magnitude. Though this paper was a methodical paper, we were able to do neural recordings in the cerebellar molecular layer in the rat from climbing fibers and mossy fibers.

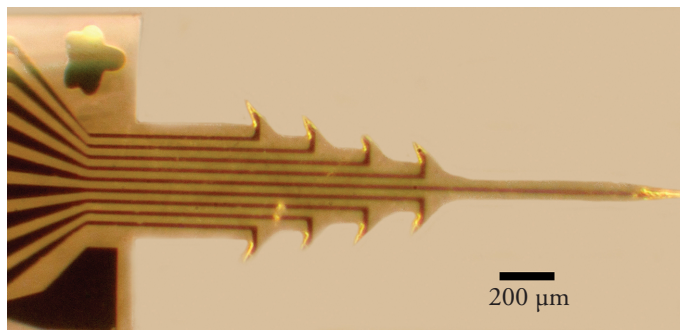


Figure 7.1: A photo of the μFoil neural probe. Modified from **Paper I** with the permission of the publisher.

My contribution to this paper was that I formed the studie on Pt-Black, I electroplated the electrodes with Pt-Black, assisted during the implantations and the recordings sessions and wrote the paper.

7.2 Paper II

Influence of Probe Flexibility and Gelatin

This paper examines the biocompatibility of the placement of the probes in either a rigid way or a flexible way and if gelatin could increase the biocompatibility of the probes. The probes implanted were fabricated in SU-8 and were a basic Michigan type probe with a thickness of 7 μm , a width of 140 μm and a length of 2 mm. In order to implant the probes, it was necessary to stiffen them, which was done by the attachment of 50 μm diameter stainless steel wires using sucrose, while some probes were also embedded in a 5-10 μm gelatin matrix. The probes were then implanted either in a rigid way, where the flat side of the probe was in the sagittal plane, or a flexible way, where the flat side of the probe was in the coronal plane. The probes were implanted for six weeks before the animals were sacrificed, perfused, the probes removed and the brain sliced and analyzed. The analysis looked at two different regions of interests (ROI) around the probe: either 0-50 μm around the probe (inner ROI) or 50-200 μm around the probe (outer ROI). For the astrocytic response we saw that there was a significant decrease in response at the inner ROI for the probes implanted in the flexible way, while for the outer ROI we saw no difference. We were not able to see any significant difference in the amount of activated microglia between the different orientations. However, we saw a significant decrease in activated microglia for the gelatin embedded probes.

My contribution to this paper was that I initiated the project, fabricated the probes, wrote part of the paper and reviewed the article prior to submission.

7.3 Paper III

μ -Foil Polymer Electrode Array for Intracortical Neural Recordings

This paper is a continuation of **Paper I**, where we have an updated design, the fabrication protocol was updated and there was a change in material of the probe, see **Fig. 7.2**. For this paper the probe was designed for the primary somatosensory cortex area (S1) of the rat brain. The probe had 13 electrodes with an area of 10 μm^2 and one reference electrode with an area of 7500 μm^2 on the surface of the brain. All electrodes were fabricated in gold with an adhesion layer of titanium, and all electrodes were platinized with Pt-Black. The platinization of the probes lowered the impedance by almost two orders of magnitude. The newly developed protocol excluded the use of HF, which makes the fabrication easier and safer. The material was changed from SU-8 to durimide, a sort of polyimide, because SU-8 was too brittle, and after the change the yield of working probes was increased. We were able to do recordings of evoked potentials and we could not detect any overhearing between the different electrodes.

My contributions to this paper was that I was part of the new design of the probes and the platinization of the probes, I assisted during the implantations and the recording sessions, I performed the impedance analysis of the electrodes, analyzed if there were any chromium left after the removal of the adhesion layer, analyzed and calculated the SNR of the electrodes and wrote most of the paper.

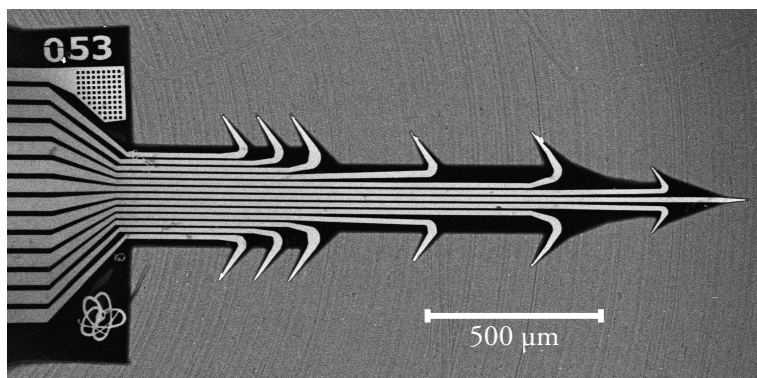


Figure 7.2: A SEM of the μ Foil probe used in **Paper V**. Modified from **Paper V** with the permission of the publisher.

7.4 Paper IV

Altered nociceptive and tactile input to primary somatosensory cortex during an episode of TRPA1 mediated hyperalgesia – implications for assessments of pain in animals

In this paper induced hyperalgesia in awake free moving animals was studied to see if mustard oil could be used as a correct pain model. μ Foil electrodes, the same version that was used in **Paper III**, were implanted into the somatosensory cortex in rats. An inflammation was induced on the paw of the animals using mustard oil. By stimulating the animal both mechanically and with a CO₂ laser at different time periods the effect that the hyperalgesia, response to the laser induced stimulation, and the allodynia, response to the tactile induced stimulation, had on the cortex was recorded. Both primary (stimulation on the area exposed to mustard oil) and secondary (stimulation on the area next to primary area) hyperalgesia and allodynia were seen in the experiments, showing that mustard oil can be used as a pain model in awake free moving animals.

My contribution to this paper was that I helped designing the probes, developed the method for using an ACF tape to connect the probes to the circuit boards, embedded the probes in gelatin, I assisted during the implantations and the recording sessions, I wrote parts of the manuscript and reviewed the manuscript.

7.5 Paper V

A polymer neural probe with tunable flexibility

In this paper a new material for the μ Foil probe was developed, OSTE+. In order to find the best blend of OSTE+, several different blends of OSTE+ were fabricated and characterized using DCS as well as DMTA. The final blend had a Young's modulus (the flexibility) of 1.9 GPa at 10 °C and 30 MPa at 40 °C with a T_g at 39 °C, which can be seen in **Fig. 7.3**.

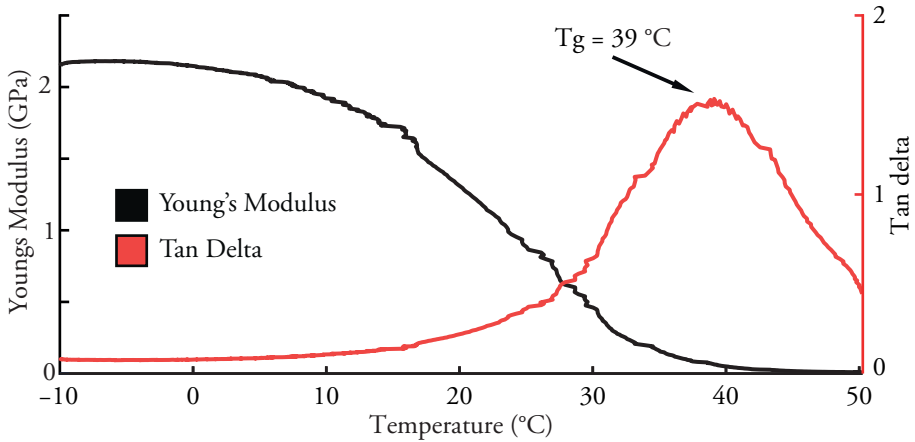


Figure 7.3: A graph of Young's modulus and Tan Delta vs Temperature for the OSTE+ blend used in **Paper V**. Reused from **Paper V** with the permission of the publisher.

A prototype probe with nine electrodes was fabricated in OSTE+, with gold used for leads and electrodes, see **Fig. 7.4** for an image of the probe. The thickness of the final probe was 35 μm . Even though that the probe was one of the most flexible probes made for intra cortical in the world, we were still able to implant it into 0.5% agar at 40 °C without using any stiffening support, and the probe was cooled using only a peltier element.

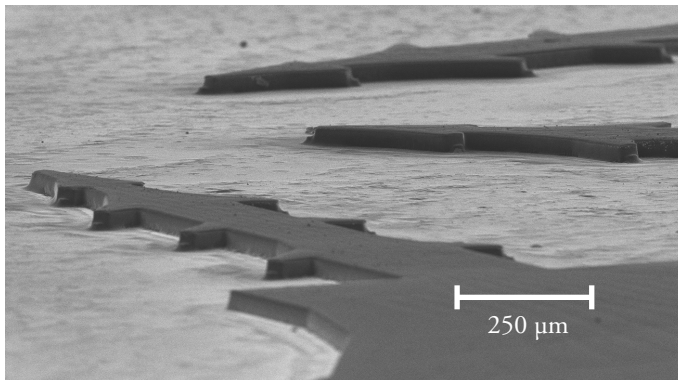


Figure 7.4: A graph of Young's modulus and Tan Delta vs Temperature for the OSTE+ blend used in **Paper V**. Modified from **Paper V** with the permission of the publisher.

My contribution to this paper was that I started the project and managed it, I did the formulations of the OSTE+, fabricated the bars of OSTE+, performed the DSC measurements, fabricated the prototype probe and wrote the paper.

7.6 Paper VI

Biocompatibility of a polymer based on Off-Stoichiometry Thiol-Enes + Epoxy (OSTE+) for neural implants

Since OSTE+ is a newly developed material there were no biocompatibility studies performed on it. Therefore in this paper we analyzed the *in vitro* biocompatibility using MTT assays as well as analyzed eventual leakage of constituents using gas chromatography mass spectroscopy (GC-MS) and liquid chromatography mass spectroscopy (LC-MS).

To perform the tests extracts of OSTE+ had to be created. The extracts were made by placing both the OSTE+ bars that had been in water for a week (OSTE+_{H2O}) and the regular OSTE+ bars into vials with clean water. The vials were then kept in motion at 37 °C for 72 hours. Extracts of OSTE+ and OSTE+_{H2O} were compared to extracts made from polyimide and High-density polyethylene (HDPE, this acted as the positive control). The MTT assays were done on diluted extracts according to: 2.5% spiked with DMSO (this acted as the negative control) 6.25%, 12.5%, 25% and 50%. The results from the MTT assays can be seen in **Fig. 7.5**, and it is possible to see that extracts from OSTE+ are toxic for higher concentrations while OSTE+_{H2O} is not.

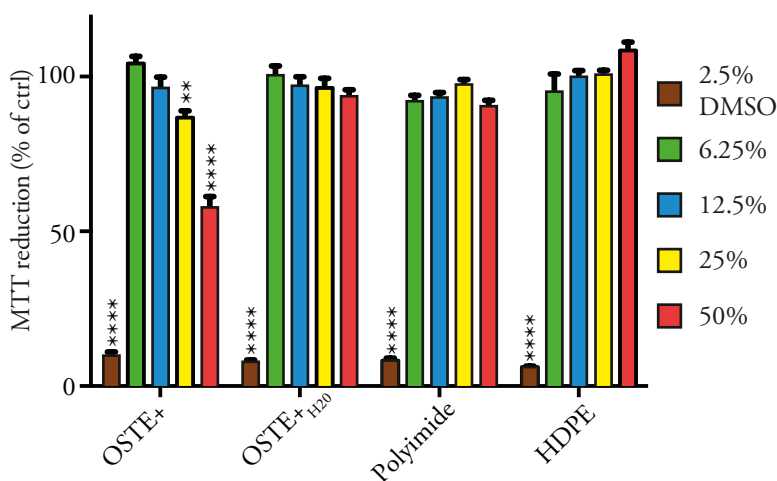


Figure 7.5: An image showing the results from the MTT assay on OSTE+, OSTE+_{H2O}, polyimide and HDPE done in **Paper VI**. The percentage represents how much extract that the medium is spiked with, e.g., 25% = 25% extract and 75% medium. DMSO was used as a negative control. It is clear that OSTE+_{H2O} is non toxic while OSTE+ is toxic in higher concentrations. Modified from **Paper VI** with the permission of the publisher.

Since OSTE+ was toxic while OSTE+_{H2O} was not, further investigations were done where the extracts and pure constituents were analyzed using both GC-MS and LC-MS. The

data from the GC-MS and LC-MS were compared to a dose-response curve made for all the different constituents. The LC-MS data showed a difference between OSTE+ and OSTE+_{H₂O}. DBN, TPO-L and diallyl was found in the extracts but when compared to the IC₅₀ value obtained from the dose-response curve no correlation was found. Hence, the toxicity has to have come either from unidentified impurities in the constituents or from synergetic effects amongst the constituents themselves. My contribution to this paper was that I planned and managed the project, developed and fabricated all the OSTE+ samples, performed the GC-MS analysis, analyzed the LC-MS data and wrote most of the paper.

7.7 Paper VII

Is the flexibility of neural probes significant for the tissue response?

From **Paper VI** we saw that OSTE+ is nontoxic to cells, but the question regarding *in vivo* toxicity was still unknown. We also know that according to theory and from studies performed on silicon and polyimide probes show that a more flexible probe should be more biocompatible, but is it really true? Since there is a change in material when testing probes either in silicon to probes in polyimide the difference might be because of the material itself. OSTE+ is a material that can be made to have different values of Young's modulus (flexibility) by changing the ratio of the constituents. And a biocompatibility test between rigid and flexible OSTE+ probes should basically be a test of the same material, but with different levels of flexibility. We implanted probes that were 250 μm wide, 20 μm thick and 3 mm long constructed in silicon, polyimide, OSTE+_{Hard} (Young's modulus at 37 °C = 300 MPa) and OSTE+_{Soft} (Young's modulus at 37 °C = 6 MPa) for four and eight weeks into mice. The mice were sacrificed and perfused, the brain were stained and then analyzed.

The results show that when comparing the tissue response from silicon probes to those of polyimide and OSTE+, the silicon probes had the largest tissue response. And when comparing the tissue response between polyimide and OSTE+; OSTE+_{Soft} has slightly less tissue response than polyimide, there is no difference when comparing to OSTE+_{Hard}. But differences between OSTE+_{Soft} and polyimide are not significant and only seen as trends it is difficult to say if the more complex processing parameters when constructing neural probes of OSTE+_{Soft} is worth the extra effort.

My contribution to this paper was that I started the project and planned most of it, developed and fabricated all the OSTE+ samples, performed the DMTA tests, analyzed part of the data and wrote parts of the paper.

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