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SILICON SIEVE ELECTRODES FOR NEURAL IMPLANTS
- *IN VITRO* CHARACTERISATION & *IN VIVO* RECORDINGS -

L. Wallman[1], A. Levinsson[2], J. Schouenborg[2], H. Holmberg[2], L. Montelius[3], N. Danielsen[4] and T. Laurell[1]
[1]Dept. of Electrical Measurements, Lund University, Box 118, S-221 00 Lund, Sweden
[2]Dept. of Neurophysiology, Lund University, Sölvegatan 19, 223 62 Lund, Sweden
[3]Dept. of Solid State Physics, Lund University, Box 118, S-221 00 Lund, Sweden
[4]Dept. of Neuroendocrine cell biology, Lund University, 221 85 Lund, Sweden
Email: lars.wallman@elmat.lth.se

ABSTRACT
An *in vitro* model was developed to characterise the electrical properties of silicon microfabricated recording electrodes, using a Cu-wire mimicing a neural signal source. Phosphorous doped electrodes were used to achieve an all silicon device.
The model was used to study signal amplitude as a function of distance between the electrode surface and the signal source. Signal crosstalk to neighbouring electrodes on the chips were recorded.The crosstalk was found to be 6 dB using an external reference electrode. Improvements were accomplished with an on chip reference electrode giving an amplitude crosstalk suppression of 20 dB. It was found that the amplitude decreased by a factor of 2 at a distance of 50 µm between the electrode surface and the signal source. Sieve electrodes were also implanted in the rat sciatic nerve and following a 10 week nerve regeneration period the dorsal and ventral (L5) roots in the spinal cord were stimulated. Compound action potentials were recorded via the chip. Lower leg muscle contraction activity was also induced by stimulating the regenerated sciatic nerve via the sieve electrode.

INTRODUCTION
In order to improve present artifical extremity technology it is necessary to develop prostheses with functions more resembling the normal extremity. This may be accomplished by recording the neural traffic proximal to the amputation site and interpret the signal pattern to control the prosthesis. Several approaches to contact the peripheral nervous system have been described previously, e.g. cuff electrodes, [1], needle electrodes which are inserted into the nerve bundle, [2], and perforated sieve electrodes implanted in a transected nerve trunk through which the nerve fibres regenerate, [3]-[7].The advantage with the sieve electrode is that the electrodes are closer to the nerve fibres and should display a higher spatial solution compared to the cuff electrode and is more stable in position than the needle electrode for long time recordings. It has previously been shown that a transected nerve can regenerate through a perforated sieve electrode and restore functional connections, [3]-[7]. *In vivo* recordings of neural activity from such devices have also been demonstrated, [3]-[5].
The present study evaluates the possibility to use doped silicon as recording electrodes. One reason for choosing this material is the low degree of tissue reactions demonstrated for silicon in soft tissue, [6].
This paper describes an *in vitro* model developed to study electrical signal recording capabilities of planar phosphorous doped silicon electrodes and to determine the signal crosstalk between electrodes on the chip. The present work also presents a pilot *in vivo* study on a nerve chip implanted in the rat sciatic nerve demonstrating functional regeneration across the chip and signal transduction to and from the neural cord.

MATERIAL AND METHODS
First generation of chip fabrication
The chips were fabricated in a three inch p-doped boron type <100> silicon wafer using standard micro machining techniques with KOH-etching. The perforated areas, two mm in diameter were defined by etching from one side in KOH forming pyramid pits. The doping pattern for the electrodes, the leads and the bonding pads were subsequently defined in the silicon dioxide. All the electrodes, leads and bonding pads were phosphorous doped. This provided pick-up electrodes having a doped layer also on the sloped walls of the holes ensuring a larger recording area per electrode. The last step included an etch step from the rear side of the wafer until the right hole size was obtained. The pyramid pit size was chosen to give the desired hole size with a chip thickness of 70 µm.The chips for the *in vitro* studies were 3 mm in diameter and had 78 square holes (90 * 90 µm). The bonding pad of the chips were glued with Araldit AV 138 M (ABIC Kemi AB, Norrköping, Sweden) on a 0.6 mm thick printed circuit board onto which thin wires were soldered. The printed circuit board were connected to the chip by wires bonded directly onto the doped silicon surface.
The fabricated chips had either four recording electrodes, Fig 3 or four recording electrodes together with two reference electrodes formed as a cross between the recording electrodes and a circle around the electrodes.

Second generation of chip fabrication
The second generation of nerve chips were processed to obtain higher transparency in the sieve region of the chip and were etched using pn etch stop resulting in a 7 um thick perforated membrane. These chips were fabricated with several different

electrode configurations. Fig 1 shows a drawing of a chip with two recording electrodes together with a reference electrode in the middle. Fig 3 shows two SEM of a chip with four recordings electrodes together with two reference electrodes.

Mounting of chips for *in vivo* experiments

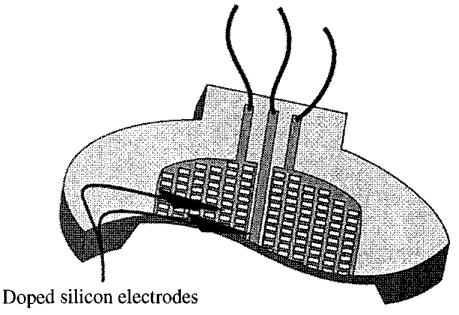

Fig 1. Drawing of the second generation of chips with a thin perforated area and a suporting ring of thick silicon

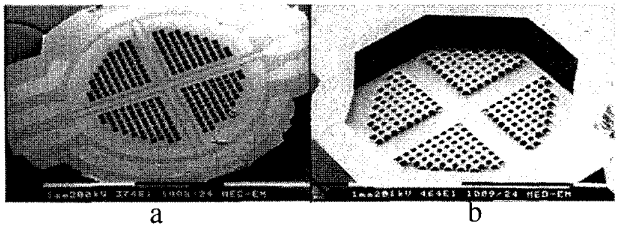

Fig 2. SEM of second chip generation. a) front side and b) back side showing the supporting silicon ring and the thin membrane area

These chips only comprised one electrode covering the whole perforated membrane. Two Teflon® insulated wires (outer diameter=300 µm, Teflon wall thickness=70 µm) were connected to the electrode by gold wire bonds via a printed circuit board. The circuit board and bonding pads were covered with a layer of epoxy glue (Araldit AV 138 M) to seal the wiring against body fluids and, furthermore, one layer of biocompatible silicon adhesive (Casco, Casco ProductsAB, Stockholm, Sweden). The use of two connections to the electrode surface offered an impedimetric control of the interconnects to the implanted chips in case of wire or bond failure during the nerve regeneration period. To be able to suture the nerves on each side of the chip two 4 mm long silicone tubes were glued with silicone adhesive (Casco) to each side of the chip.

***In vitro* model**

To mimic physiological conditions the *in vitro* measurements took place in a beaker with Ringer solution. The nerve fibre was simulated by an insulated Cu-wire with an uninsulated spot at its termination which corresponded to a node of Ranvier Voltage pulses simulating nerve pulses were applied to the wire and the Cu-wire thus acted as a firing nerve fibre. All the electrodes on the chip were connected via an oscilloscope to a computer using LabView™ (National Instruments Inc.,Austin, Texas, USA). Fig. 3 shows a schematic figure of the*in vitro* model. Simulated nerve pulses were applied to the Cu- wire and simultaneously the signal on the chip electrodes were monitored by the computer.

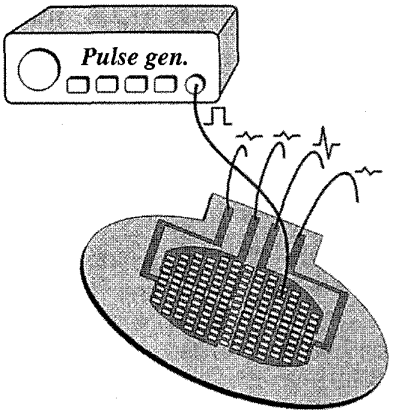

Fig 3. In vitro model where the nerve fibre is simulated with a Cu wire. The measurements were performed in Ringer solution.

***In vivo* study**

The rat sciatic nerve on the left leg was exposed and cut at the midthigh level proximal to the tibial and distal bifurcation.The proximal and distal stumps were sutured in the silicone tube 2 mm from the chip surface. The two wires connected to the chip were sutured in a muscle and placed subcutaneously onto the back of the rats.

Ten weeks after the surgery the rats were decerebrated under halothane anaesthesia. A laminectomy of the vertebrae Th13 - L2 was made and the dura mater was incised and split open. The dorsal roots L5 and L2 and the ventral root L5 were dissected and cut. The distal ends were hooked on bipolar silver electrodes for electrical stimulation. Nerve activity in the sciatic nerve was recorded via the chip electrode and also from a bipolar electrode placed about 10 mm distal to the chip electrode. A reference electrode was placed either in the skin of the neck, the lower back or the tail. Fig. 4 shows a schematic picture of the spinal cord together with the sciatic nerve which had regenerated through the nerve chip.

RESULTS AND DISCUSSION

***In vitro* study**

Fig. 5 shows the coupling of a signal (simulated nerve signal) from the end of the Cu-wire versus the distance to the electrode surface (first generation chip). In Fig. 5a the recorded signal amplitude relative an external reference electrode in the solution is plotted for two recording electrodes, the correct electrode and a neighbouring electrode, together with the amplitude of the reference electrode. As shown in Fig. 5a the signal of the neighbouring electrode and the reference electrode displayed almost the same common mode signal. Therefore a differential

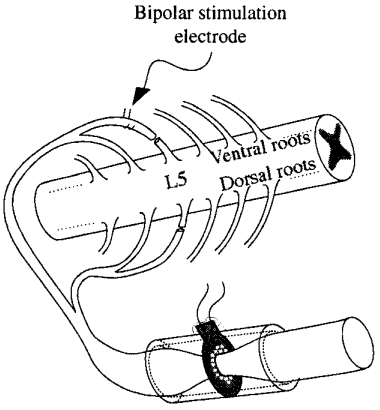

Fig 4. Schematic drawing of the preparation for the *in vivo* recording. The dorsal and ventral roots of L5 were cut and the distal ends were hooked onto and stimulated by a bipolar electrode.

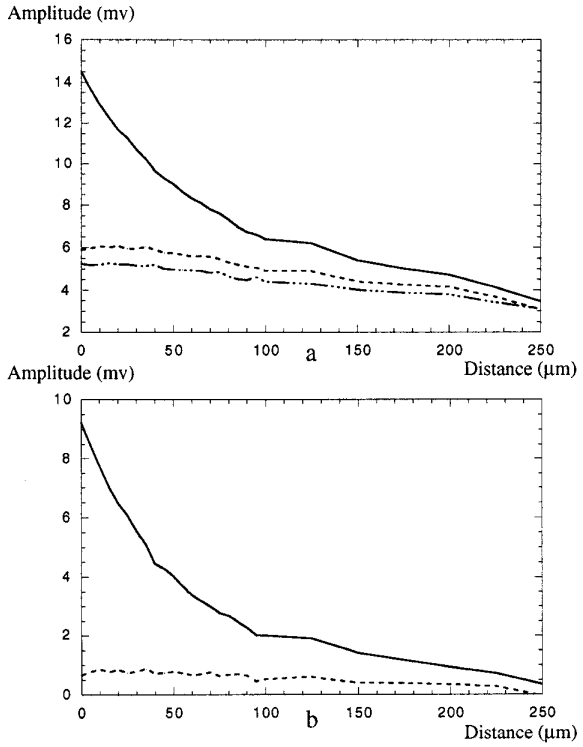

Fig 5. The signal from the electrodes plotted versus the distance between the Cu-wire and the recording electrode (solid line), neigbouring electrode (dashed line) and on chip reference electrode (dashed/dotted line). a) recordings versus the external reference electrode in the solution and b) versus the on chip reference electrode.

measurement mode was used. In Fig. 5b the amplitude is plotted relative the on-chip reference electrode for the correct and a neighbouring electrode. Close to the electrode surface this gave an increase in crosstalk suppression from 6 dB (external reference electrode) to approximately 20 dB (on chip reference electrode). The figure also shows as expected that the best cross-talk suppression was achieved when the Cu-wire was close to the surface of the chip. In the case of an *in vivo* application where the typical inter nodal distance of a regenerated nerve is approximately 100 µm and numerous nerve fibres regenerates through each hole, there is a high probability that several nodes of Ranvier would be closer to the chip electrode than 50 µm.At a distance of 50 µm between (the longest inter nodal distance to a Ranvier node in the regenerated nerve) the Cu-wire and the electrode surface a crosstalk suppression of 13 dB was accomplished.

In Fig. 6 the Cu wire was scanned across the surface at a fixed a distance (10 µm) from the chip surface and the signal from one recording electrode was monitored. Fig. 6a shows the amplitude of the recorded signal versus the external reference electrode in the solution. The geometry of the recording electrode is marked in the Figure by a solid line and the position of the neighbouring electrodes are dashed. Fig. 6b shows the same scan with the signal from the recording electrode measured relative the on-chip reference electrode. The light areas in the figures correspond to a high amplitude of the signal and the dark areas corresponds to a low amplitude of the signal.

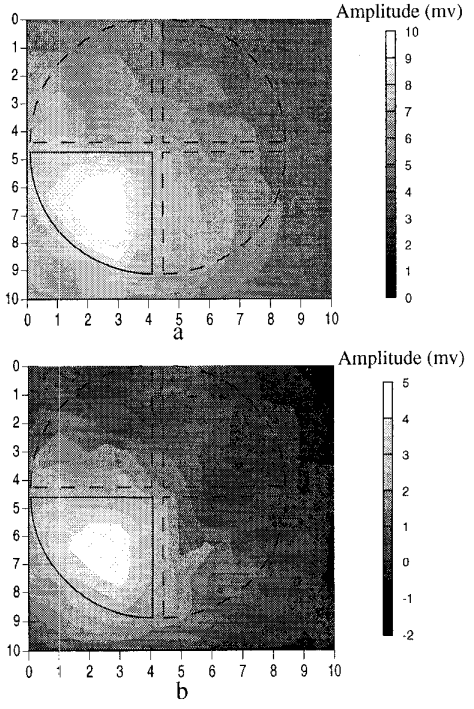

Fig 6. The recorded signal from one electrode when scaning with the Cu-wire over the entire surface at a distance of 10 µm

In vivo study

In one of the implants, the connected wires were intact. In this rat, stimulation through the chip electrode at low intensity (pulse trains of 100 Hz, 200 ms, 30 μA) evoked a muscle twitch in the flexor digitorum longus muscle indicating that sciatic motor nerve fibres had regenerated through the chip and reinnervated lower leg muscles. Stimulation of the dorsal and ventral root of L5 with a small current (2 μA, pulse duration 0.2 ms) evoked a short latency compound action potential in the sciatic nerve which could be recorded from the chip electrode, Fig. 7. This compound action potential had peak latency of about 2.5 ms, corresponding to a conduction velocity of about 30 m/s. In addition, stimulation of the dorsal root of L5 at a higher current (10 μA) evoked a compound action potential with a longer peak latency (8.5 ms), possibly mediated by Aδ fibres. It should be noted that the location of the reference electrode did not influence the amplitude and latency of the compound action potential as recorded from the chip electrode. Furthermore, even intense stimulation of the L2 dorsal root did not evoke detectable nerve activity from the chip electrode nor from the recording electrode distal to the chip. Since the L2 root normally contributes very little or nothing to the sciatic nerve, this finding indicates that only stimulation of the spinal roots contributing to the sciatic nerve could evoke a compound action potential detectable by the chip electrode. It thus seems likely that the compound action potentials in both sensory and motor myelinated fibres recorded by the chip electrode reflected impulses in the nerve fibres which had regenerated through the chip.

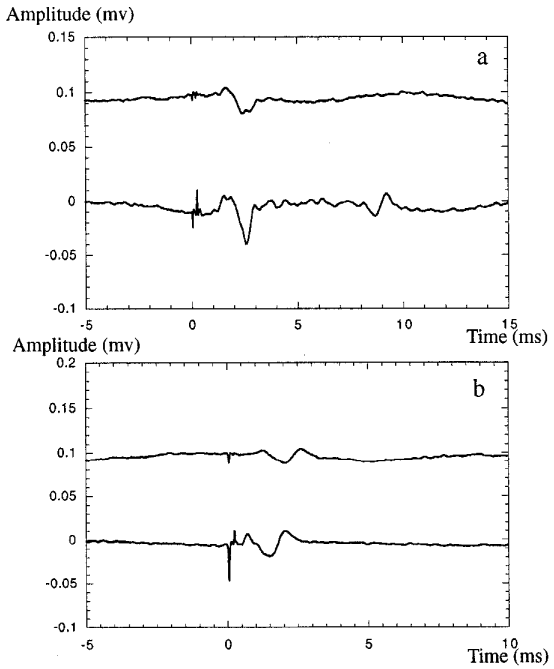

Fig 7. *In vivo* recordings from a) dorsal root L5 and b) ventral root L5.

CONCLUSION

An *in vitro* model has been developed to characterise the signal recording capabilities of sieve electrodes before implantation. This will make the animal work more optimal and keep the number of implants needed at a minimum.

At a distance of ≤ 50 μm between the chip and the signal source an inter electrode crosstalk suppression >13 dB was measured *in vitro*. It is thus concluded that in an *in vivo* situation it should be possible to discriminate the signals from different chip electrodes such that each electrode region records activity from different fascicle regions in the nerve.

It is possible to use an all silicon neural interface with phosphorous doped electrodes to obtain *in vivo* recordings of nerve signals from the rat sciatic nerve.

Further studies will focus on *in vivo* measurements with nerve chips having multiple electrodes. Also, the *in vitro* model will be used to further optimise the chip and electrode design to obtain better signal coupling and improved crosstalk suppression.

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