Neuro-electronic interfaces: 2D and 3D multi-micro electrode systems and cultured multi electrode plates

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Abstract - Force recruitment experiments with a two-dimensional 24-fold multi electrode resulted in selective stimulation of 10 motor fibres exclusively, out of the total of 69 motor fibres of Extensor Digitorum Longum muscle, in the rat peroneal nerve (this means a device efficiency of 10/24=42%). The University of Twente three-dimensional 128-fold silicon micro-electrode device is estimated to be able to selectively stimulate 43 motor fibres.

Alternatively, fabrication of a two-dimensional 128-fold multi electrode device in LIGA (Lithography, Galvanic shaping) technology was performed.

Microfabrication technologies appear to be an important tool for neuro-electronic interfacing, but for even more efficiency of interfacing, silicon (or LIGA) multi electrode devices will probably be not yet small/selective enough, as one would need too many electrodes to be inserted into a nerve fascicle. Therefore, it is discussed how in-vitro-neuron cell-cultured MEP’s (Multi Electrode Plates), might enhance efficiency in future implantable devices.

Keywords: neuro electronic interfaces, neurotechnology, electrical stimulation, selectivity, microfabrication, cultured neurons

Introduction

The availability of large-scale (many electrode sites), selective neuro electronic interface devices is the most essential prerequisite for selective artificial stimulation of peripheral nerve motor fibres, for possible future rehabilitative neuromuscular control of extremities (spinal cord lesion patients).

Selective stimulation means that single motor fibres are activated endoneurally, to control single muscle units, thereby enabling graded control of muscle force and the postponement of muscular fatigue.

As the number of motor fibres in an average fascicle is in the order of a few hundred and the architecture of a fascicle is not precisely prescribed by nature, it is evident that the best possible approach at present is to employ the possibilities of microfabrication techniques for the design and construction of a redundant number of micro electrodes in 3D arrays.

Earlier calculations and experiments in rat peroneal nerve, to control the Extensor Digitorum Longus (EDL) muscle, yielded that electrodes should be separated no more than 268 micrometer apart for optimal selectivity [1, 2]. (The peroneal nerve has about 350 α motor fibres [3, 4, 5] which control four muscles, the hallucis, peroneal, tibial and EDL muscle. Out of these, a number of 69 +/- 15 control the EDL muscle [6]).

This paper reports on the selectivity, experimentally obtained with ‘hand-made’ 24-fold 2D arrays, of which electrodes were spaced at 120 micrometer, in rat peroneal nerve and EDL muscle. Also, the design and the realisation steps of a 128-fold 3D array in silicon- and glass-technology are briefly mentioned, as well as the fabrication of a 2D 128-fold array in silicon- and LIGA (Lithography and Galvanic shaping) technology.

Special attention is given to the efficiency of devices, i.e. the ratio (percentage) of ‘successful’ electrodes, contacting a single motor fibre, and the total number of electrodes in the device.

Finally, it is discussed whether an alternative way of interfacing, namely employing cell cultures on electrode substrates, will lead to higher efficiencies.

METHODS

Five acute experiments were conducted on five Wistar rats. A two-dimensional wire-micro electrode array (see figure 1, lower part) was inserted into the intact peroneal nerve through an epineural incision [7,8]. The electrode array consisted of 6 rows of 4 NiCr-wire electrodes each. In the experiments all 24 electrodes were used (2 rats), or 17 (one rat), 14 (one rat), or 8 (one rat). Diameter of the wire electrodes was 25 µm; inter electrode spacing was 120 µm. Rectangular depolarizing current pulses of 100 µs duration were generated in order to stimulate α-motoneurons of the EDL. The elicited twitch forces were measured under isometric conditions with a Harvard Isometric Force transducer (model 373, 100 gram), with an A/D accuracy of 0.5 mN. For each of the electrodes in the array a recruitment curve was recorded by applying stimuli with increasing amplitudes at 1 s intervals; stimulus current step size was 0.1 µA. The EDL was allowed 2 minutes of rest between recruitment curves for consecutive electrodes. The peroneal nerve was kept moist by applying Ringer’s solution at regular intervals.
RESULTS

Recruitment curves obtained with 2D wire multi electrode

For each of the electrodes in the array the corresponding twitch-force recruitment curve was recorded from the EDL muscle. Figure 1 shows 4 out of 24 recruitment curves from one animal. Only twitch-force maxima exceeding 4.9 mN (0.5 g) are shown, since this value represents the lowest measurable force with the force transducer employed [8]. The curves were recorded sequentially, from electrode 1 to electrode 24. Long term stability was carefully monitored [8]. Note that the curves are plotted on a double-logarithmic scale. Recruitment curves from different electrodes have different shapes in the low-force range and many have different threshold (most important for selectivity).

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Figure 1. Twitch force recruitment curves of rat EDL muscle stimulated by a 24-fold 2D electrode array (see below), electrode spacing is 120 µm. Scales are log-log. Vertical scale ranges from 0.1 to 100 grams, horizontal from 1 to 100 µA. Only 4 curves of the 24 are shown. See [8] for more detail.

Selective recruitment of motor units

The accuracy with which force has been measured is 0.25 gram (2.45 mN), and the force noise floor lies at 0.5 gram (4.9 mN). This means that we could not measure motor unit forces below 0.5 gram, and that measured forces within the same 0.25 gram wide bins are identical.

A number of 10 distinct motor units were found to have been contacted at threshold by 24 electrodes [8]. This implies an efficiency of 10/24=42% (while in a statistical model, we expected an efficiency of 8/24=33%). This result was obtained by classifying/assigning the first force level (but above the noise level of 0.5 gram) to the appropriate bin (bin width 0.25 gram). A number of 10 bins resulted, centered at 0.5 gram (8 electrodes), 0.75 (4 electrodes), 1 (3 electrodes), 1.25, 1.5, 2.25 (each 1 electrode), 2.75 gram (2 electrodes), 3, 3.75 (each 1 electrode) and 5 gram (2 electrodes). Subsequent analysis in each bin reveals that, for example in the first bin, electrodes 4, 5, 7, 8, 12, 15, 16 and 23 are involved. Of these numbers, 4 and 5 are neighbor electrodes, so it is more likely that these two electrode stimulate the same motor units. Also, 7 and 8, as well as 15 and 16 are neighbors. However, 23 and 12 are farther apart, implying that the stimulated threshold motor units are different (and maybe composed of a number of units with strengths below 0.5 gram). This way, further analysis reveals that the number of distinct units contacted, increases from 1 to 5 in the first bin, and from 10 to 20 for the total array.

Microfabrication of 3D silicon/glass device

The fabrication of a 3D silicon/glass-based multi micro electrode (figs 2 AND 3) involves a large number of challenging steps [9], not only for the 'brush' itself, but also for the control electronics chip [10] and the contacting technology (between electrode-brush and control chip) [11]. The chip has been completed, the solder bump contacting technology (flip-chip technology) is ready, and nearly all fabrication steps of the brush are successful now.
Figure 2. Schematic overview of a CMOS chip carrying a 3D array of 128 microelectrodes, to be inserted into a nerve. For dimensions, see Fig. 3.

Figure 3. Dimensions of multi electrode array mounted on CMOS chip. Electrode needles are 250, 425 and 600 µm long, their spacing is 120 µm.

**Microfabrication of 2D silicon/LIGA device**

An alternative way to fabricate a 3D device would be to combine silicon- with LIGA-technology (Lithographie, Galvanoformung, Abformung) . Nickel needles are grown from a seed layer through narrow channels in 200 µm PMMA (polymethylmethacrylate) (Fig. 4).

Figure 4. Array with 200 µm tall needles, realized with aligned X-ray lithography (LIGA) on silicon substrate with 8 µm Cu interconnection wiring.

Although the nickel growth process is still delicate, this combination of techniques may be useful for fabrication of neuroprostheses. Also, the length of the needles must be increased to about 500 µm [12]. The advantage of the LIGA method may be that electrical isolation of the needles is easier, it will reduce the number of process steps.

**DISCUSSION**

**Fabrication**

Silicon/glass and silicon/LIGA fabrication seem good candidates for fabrication of 2D and 3D devices. Silicon/glass technology has the advantage of high aspect ratios, sufficient length of needles and different lengths of needles in the same device. Disadvantage is the 3D nature of many of the process steps, and the large number of steps.

Silicon/LIGA technology reduces the number of steps, but has as disadvantage the need for synchrotron radiation facilities. Also, the present limit of the electroplating process to 220 µm long/15 µm thick nickel needles has to be extended to a needle length of about 500 µm [12], for a useful neuroprosthesis application.

**Future developments: cultured multi electrode plates as more efficient neuro-electronic interfaces**

If selective contact of one-electrode-one-fibre is to be obtained, one has to bring an electrode close to one of the nodes of Ranvier of a fibre. The only 'garantee' for that in a 'random' population of fibres is the use of a redundant number of electrodes in the nerve. For example, the peroneal nerve of the rat controls four muscles. One of these is the Extensor Digitorum Longum (EDL) muscle, with 69 motor units [6]. Assuming a random transverse topology for the position of the parallel EDL motor fibres in the peroneal nerve/fascicle, our multi-electrode device with 128 electrodes on top of 15-40 µm thick tapered needles is expected to have an ‘efficiency’ of 43 motor fibres to be contacted selectively, that is a model efficiency of 43/128≈34 %. (see before, results section). This number, and extrapolations from experiments with the 24-fold 2D device, indicate that control of all 69 fibres indicate the need for much more than 128 electrodes, in the same volume. This will be hard to realize by microfabrication techniques, but it will also become harder to insert such a large volume of needles in the nerve trunk. Therefore, increase of the ‘efficiency’ of the device would be welcome.

Increase of efficiency is possible by a different approach. Instead of 'putting in more and more electrodes', one could reverse the process: let nerve fibres (or collaterals from motor fibres) grow towards and on electrodes. If each electrode contacts one fibre, efficiency is 100%.
A different, pioneering approach in this respect is that of Tatic-Lucic et al. [13], in which single hippocampal cell somata are contained in silicon well-electrodes. Instead of catching one cell in a well, our approach will be to cover each electrode site (in a planar array, or Multi Electrode Plate, MEP) with a small local network of neurons. Then, each in vitro cultured network per electrode site will serve as ‘target’ for one fibre-collateral ‘in vivo’.

We have recently started this two-step approach. The first step is that electrodes are covered by cultured nerve networks, by growing cells in a controlled way on adhesively patterned Multi Electrode Plate (MEP). Patterning is necessary to confine the local network to the electrode area. We have produced MEP’s with 60 electrode sites and HIPEC (a polysiloxane polymer) coating, on which cortical cells have been grown successfully and were electrically active over long periods. The HIPEC layer was in this case unpatterned, and covered uniformly with laminin or PEI (polyethylenimine) for uniform neural cell adhesion [14]. An example of a similar MEP, but now with a chemically patterned surface layer, is given in figure 5. The ITO (indiumtinioxide) electrode sites are positioned at the crossings of neurophilic pathways (APS, 3-aminopropyltriethoxysilane, coupled to SiO2), separating neurophobic square areas (TMCS, trimethylchlorosilane, coupled to SiO2). Experiments with neural culturing on this patterned plate are underway.

Figure 5. Microphotograph of part of multi electrode plate (MEP), showing 9 ITO electrode sites, of which 6 are wired to the outside world. Electrodes are covered by silicon oxidered silicon nitride-silicon oxide sandwiched layers, except at electrode sites (black circles). Streets are neurophilic pathways (APS coating), large squares are neurophobic areas (TMCS coating). APS and TMCS are silanes, coupled to the upper siliconoxide layers.

REFERENCES