Resorbable PLGA Microneedles to Insert Ultra-fine Electrode Arrays in Neural Tissue for Chronic Recording

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1 OBJECTIVES

It has been shown that the mechanical rigidity of neural implants is a key factor that causes the formation of scar tissue around the implant. Clearly, this reduces performance and lifetime. Therefore, recent work has focused on very compliant, polymer-based implants (Weltman, 2016). However, such implants need a temporary reinforcement to aid their insertion in neural tissue (Lecomte 2018).

Recent work has even indicated that it is possible to keep polymer thin-film neural electrode arrays in close contact with neural tissue over chronic timescales, without the formation of scar tissue (Zhou 2017). To achieve this, injection of an electrode array suspended in liquid was used, which is hard to upscale to higher electrode counts and relatively cumbersome.

In this work, we are investigating the use of microneedles fabricated out of short-chain, fast resorbing polylactic-co-glycolic acid (PLGA, Purasorb PLDG 5002A) as a temporary reinforcement.

This technique improves the existing arrays, injected using a capillary, in terms of controllability and upscalability to larger electrode counts.

Therefore, the objective was to device a fabrication process, that allows to micromachine needle-shaped PLGA structures and to embed ultra fine electrode arrays in those needles. A second objective was the long-term in vivo testing of the electrode arrays in rats.

2 METHODS

The electrode arrays are fabricated by a lithography –based processing technology published earlier (Ceyssens, 2015). The process was adapted to reduce the implant thickness to only 1 µm, yielding an ultra-flexible implant backbone.

The resulting arrays were designed to contain a linear array of 16 iridium oxide electrodes, aimed at single neuron recording. The electrodes are 15 micrometer in diameter. Polyimide (HDMicrosystems PI2611) is used as insulation material. After fabrication, an Omnetics Nano connector is attached to connect an external amplifier during testing. The micromachined wires between the connector and the electrodes are only 10 µm wide.

Separate microneedles for support are fabricated out of a short chain PLGA, that resorbs over a period of 2-3 weeks after implantation. Molding or picosecond UV laser machining is used. The needles have a cross-section of 0.35 x 0.25 mm². In a final step, the needles are bonded to the electrode array using thermocompression.

For in vitro testing, the needle arrays were implanted transdurally in the right sensorimotor cortex of four rats, 4 mm right from Bregma. At intervals, at least one week apart, the rats were anesthetized using usoflurane. The spontaneous spiking activity was recorded. In a second test, biphasic electric pulses (800 µA amplitude, 0.1 ms per phase) were applied subcutaneously to the left forepaw to evoke a muscle contraction. Meanwhile, the presence and strength of the evoked potential in the brain was monitored.

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To quantify the electric recordings, the results were filtered (FIR HP filter, 200 Hz stopband, 250 Hz passband) and a spike counting algorithm based on a moving window was adopted. Spike threshold was set to 4 standard deviations below the window average. To quantify the evoked potential, a FIR HP filter with 10 Hz stopband was used and post-stimulation peak-to-peak (PTP) values were recorded. Also, the RMS ratio after/before stimulation was determined.

After approximately four months, the rats were sacrificed and a histological evaluation of the implantation sites was performed (GFAP and NeuN staining).

3 RESULTS

3.1 Fabrication and in Vitro Test

The fabrication process yielded about 50% devices without defects (Figure 1: example from early fabrication run). The arrays are 1 µm thick in total, and the metal tracks are 4 µm wide, with 3.5 µm of insulating PI on every side. An example needle is shown in Figure 1. The measured electrode impedance was around 200 kOhm at 1 kHz.

As an insertion test in Agar gel proved insufficient adhesion between the implant backbone, a second version was fabricated in which the backbone was squeezed between two PLGA needles of half the thickness. This proved to have sufficient adhesion.

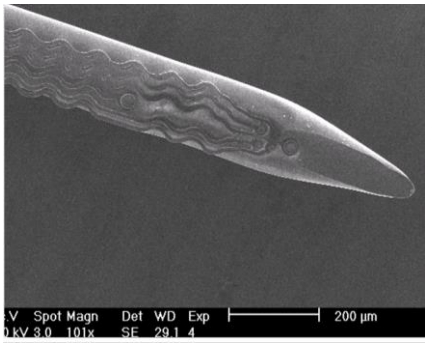

Figure 1: PLGA needle with electrodes.

3.2 In Vivo Testing

The implantations (Figure 2) went as planned. No wound inflammation or abnormal behaviour of the animals was observed.

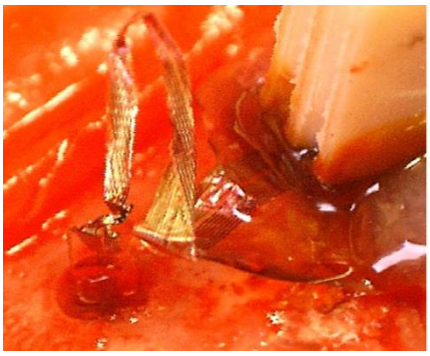

Figure 2: Implantation, showing transparent PLGA microneedle in burr hole (lower left), connecting cable and Omnetics connector (upper right).

About 50 days after implantation, the headstage of rat 3 disconnected from the skull, after which no measurements were possible on the animal. In all other animals, it was possible to observe signals throughout the duration of the experiment.

In general, action potentials and evoked potentials could be clearly observed over the entire course of the 4 month time span. There were no channels dropping out. A typical measurement of spontaneous action potentials (4 months after the start of the experiment) is shown in Figure 3.

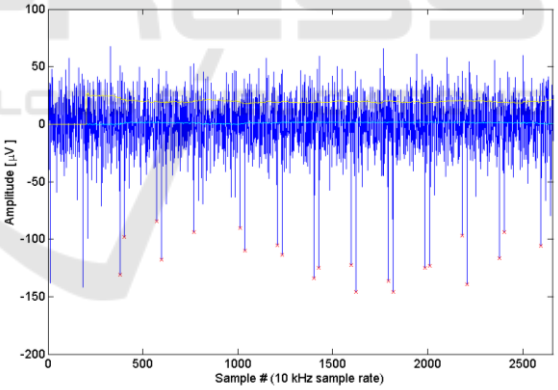

Figure 3: Sample of observed spontaneous spikes (rat 4, 4 months after implantation). Red crosses show peaks detected by the algorithm used.

Figure 4 shows an example of an evoked potential recording, also after 4 months.

The number of observed spontaneous spikes per second, and the size of the evoked potential are shown in Figure 5. The average PTP value (over all channels) is around 2 mV, and about 70 spikes per second can be seen per channel. Especially the latter measurement shows a relatively large variation, though.

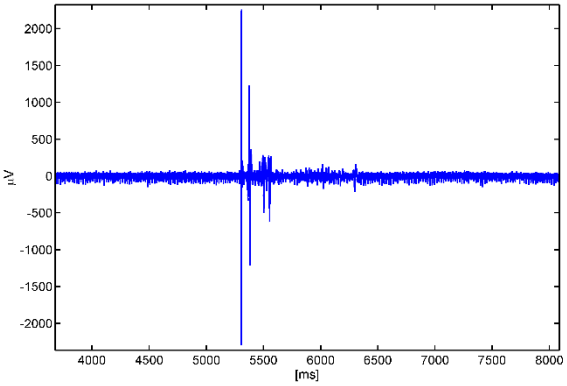

Figure 4: Example of evoked potential recording (rat 4, channel 11, 4 months after implantation). The largest peak is the stimulation artifact, which is followed by a train of relatively low-frequency responses.

3.3 Histology

Due to the shallow insertion depth, it was not possible to remove the rat brains for histology without pulling out the implant, though it was still possible to inspect the glial scar.

Histology revealed that there was still limited glial scarring present. The remaining scar is a factor 4 – 6 times smaller in cross sectional area than the original size of the PLGA needle. An example is given in figure 6. In later work, a full analysis of all histological data will be included.

4 DISCUSSION

Briefly, though no full integration (i.e. the electrode array floating in between neurons without any scar in between) with the neural tissue was achieved, the observed astrocytic scar was minimal. Viable neurons were seen to be present inside the volume that used to be taken up by the resorbable structure.

Qualitatively, the measurements were stable and a similar response was seen over the four months of the experiment.

Quantitatively, the number of spikes per second and the peak-to-peak values of the evoked potential was seen to vary about a factor of 2 in between experiments. As there is no clear upwards or downwards trend and this variation is even present between measurements just a few days apart on the same rat, we can assume this is likely due to inherent variability in the experimental setup.

This includes the depth of the anesthesia, the exact positioning of the electrode used for stimulation and natural variations.

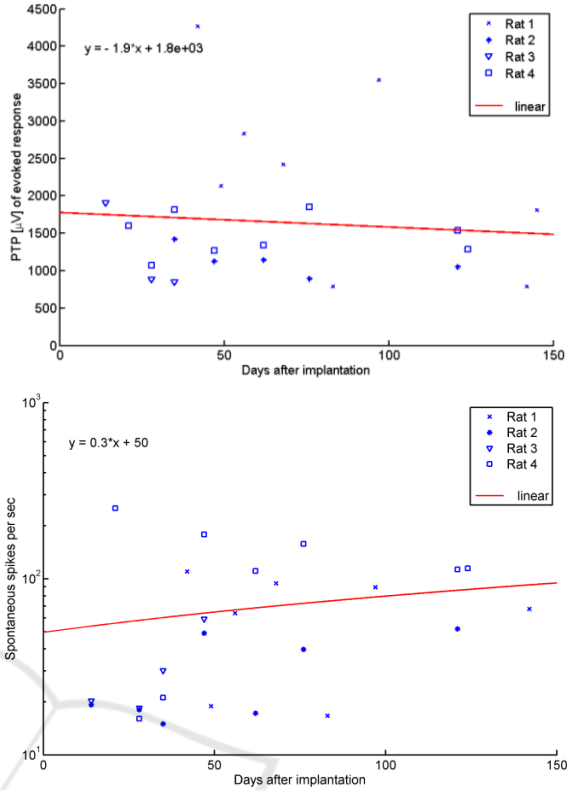

Figure 5: Top: measured PTP value (per rat, averaged over all 16 channels) of the evoked potential over time. Bottom: Number of spontaneous spikes per second.

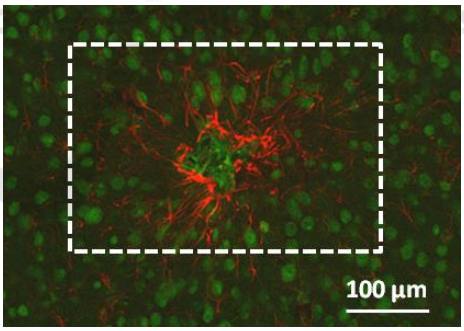

Figure 6: Scar after end of experiment. Red: GFAP stain. Green: NeuN stain, revealing the presence of viable neurons up to 100 µm close the center axis of implantation. The dashed rectangle indicates the approximate boundary of the original PLGA needle.

5 CONCLUSION

We conclude that this method of inserting ultra-fine electrodes arrays is practical and yields only minor tissue damage. Combined with the polyimide-based neural electrode array presented, we were able to

record evoked potentials and action potentials for at least four months.

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