ULTRA HIGH-ASPECT-RATIO NEUROPROBE: 5-µm-DIAMETER AND 400-µm-LENGTH NEEDLE DETECTS ACTION POTENTIALS *IN VIVO*

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ABSTRACT

This paper reports 5-µm-diameter and 400-µm-length ultra high-aspect-ratio needle electrodes for neuronal action potential recordings *in vivo*. The needle-electrode was vertically assembled on a 1 × 1 mm^2 block module of silicon by vapor-liquid-solid (VLS) silicon growth technology for ~ 7 hours. After metallization and encapsulation of the needle, the module device was packaged for *in vivo* animal experiments. The fabricated needle-electrode, which has an electrical impedance of 328 kΩ at 1 kHz, shows the smooth tissue penetration and neural recording capabilities, as demonstrated by the needle penetration and recording action potentials of a mouse's cortex *in vivo*. To the author's knowledge, this is world's smallest extracellular recording needle, which will offer numerous electrophysiological recording applications while the brain tissue damage can be reduced. As a future work, the fabricated needle module device will further be minimized and arranged in an array of high dense needles with 100% device yield, while other functionalities (drug delivery and optogenetic applications) will be integrated with the same needle array.

INTRODUCTION

Compared to other methodologies (optical and magnetic measurement), electrophysiological recording with fine needle-electrodes, which can be put around the neurons, is known as a way to offer a high spatiotemporal resolution of neuronal activity recording. By using recent advances in MEMS technology, numerous three-dimensional needle-electrode devices have been facilitated [1]. However, to realize chronic recordings of high quality neuronal signals, the diameter of the needle should be less than 10 µm, which geometry is necessary to reduce brain damage [2, 3]. Additionally, for exploring deep cell layers in the cerebral cortex, the length of the needle should be longer than 400 µm (e.g., 400 µm depth for IV cell layer in mice, II-III cell layer in rat [4], and III layer in monkey).

To overcome the issues of conventional needle geometry, here we fabricate 5-µm-diameter and 400-µm-length ultra high-aspect-ratio needle-electrodes (Fig. 1), and the tissue penetration and neuronal recording capabilities of the needle were demonstrated using a mouse's brain *in vivo*.

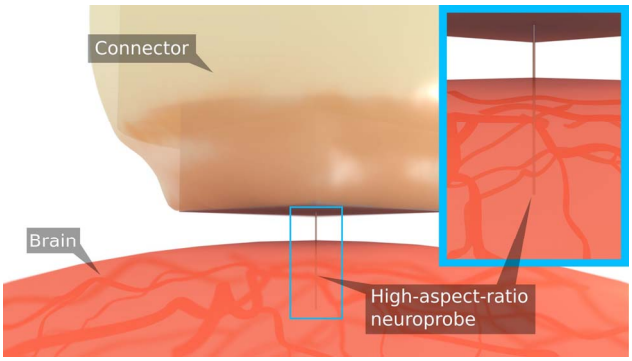

Figure 1: Concept image of the proposed high-aspect-ratio needle-electrode neuroprobe. The needle has the length over 400 µm with the diameter of < 10 µm, which can minimize the brain damage.

DEVICE FABRICATION

High-aspect-ratio needle electrode devices are fabricated by VLS silicon (Si) growth and three-dimensional microfabrication processes. We use a heavily doped Si wafer (525 µm in thickness), which acts as the vertical interconnection between the top (needle site) and the backside metals. After silicon dioxide (SiO_2) layer formation, catalytic gold (Au) was patterned by lift-off process (Figs. 2a, b). With the catalyst, VLS growth process for ~ 7 hours around 730°C was executed to grow 400-µm-length Si needles (growth rate = 1.2-1.6 µm/min) (Fig. 2c). After the VLS growth process, SiO_2 was removed by buffered hydrogen fluoride (BHF). The needle was metalized with platinum (Pt) with adhesion layer of titanium (Ti)(total Pt/Ti thickness = 200 nm) by sputtering and lift-off process (Fig. 2d). The wafer backside was also metalized with the same metal (Pt/Ti). The metalized needle was encapsulated with a biocompatible insulator of parylene-C (1 µm in thickness, Fig. 2e), while the tip section of the needle was exposed by oxygen (O_2) plasma. Finally, the wafer was diced into 1 × 1 mm^2 block modules with stealth dicing (Fig. 2f-g), resulting in approximately 500 needle-blocks taken from a 3.5 × 3.5 cm^2 wafer (~ 50% yield).

Figure 3a shows a fabricated Si block module, which consists of a 5-µm-diameter (tip section) and 400-µm-length needle-electrode at the center portion (Figs. 3c, d). For numerous animal experiments, the block module was connected to a pin connector with conductive epoxy, while the sidewall of the needle module and pin connector was insulated with insulating epoxy (Fig. 3b).

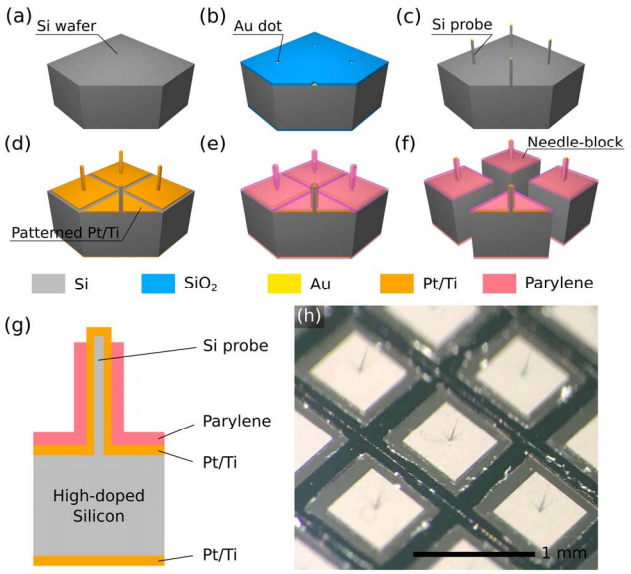

Figure 2: Batch fabrication of high-aspect-ratio needle electrode blocks. (a-c) Si-wafer oxidation, catalyst (Au) formation, VLS growth, and SiO_2 removal. (d) Needle metallization with Pt/Ti. (e) Parylene deposition. (f) Needle-tip exposure and wafer dicing. (g) Schematic of cross sectional image of a fabricated needle block. (h) Microscope image of the fabricated block devices.

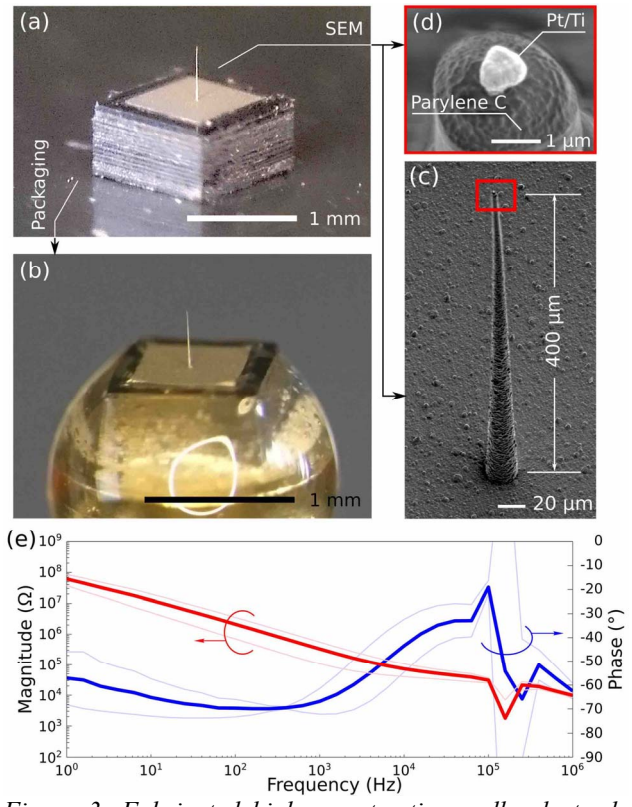

Figure 3: Fabricated high-aspect-ratio needle electrode (a) Photograph of a diced needle block. (b) The block is mounted on a pin connector. (c, d) SEM images of the needle section. (e) Magnitude (red) and phase (blue) of the electrode impedance measured in PBS at R.T. (Note: both of thin lines show standard deviation).

Since the electrode shows high impedance characteristics in PBS due to a small electrode site, the electrode impedance was reduced by plating a low impedance material of platinum-black (Pt-black) at the needle-tip. Figure 3e shows the impedance characteristics of a Pt-black tipped needle after the device package (Fig. 3c). The electrical impedance measured in PBS at 1 kHz shows 328 kΩ ± 221 kΩ (mean ± SD, n = 3, Fig. 3e), which is low enough to measure neural signals, including local field potential (LFP) (< 500 Hz) [5] and action potential (~ 1 kHz) [2].

ANIMAL EXPERIMENT

Penetration capability

After the electrical characterizations, the tissue penetration capability of the needle was demonstrated using a anesthetized mouse's brain *in vivo* (Fig. 4). Smoothness of the penetration could be tested by the deformation of the object surface. For quantitative analysis of the brain deformation, we used markers on the tissue surface by plotting blue dots (500 µm center-to-center spacing). These dots are monitored during the needle penetration. Surprisingly, due to the fine needle geometry (~ 5 µm in diameter), the needle smoothly penetrates the brain without significant tissue deformation (~ 4.3 µm deformed from initial tissue-surface). On the other hand, 20 times larger tissue deformation of 94.2 µm was observed with a conventional ~ 80-µm-diameter needle (data not shown).

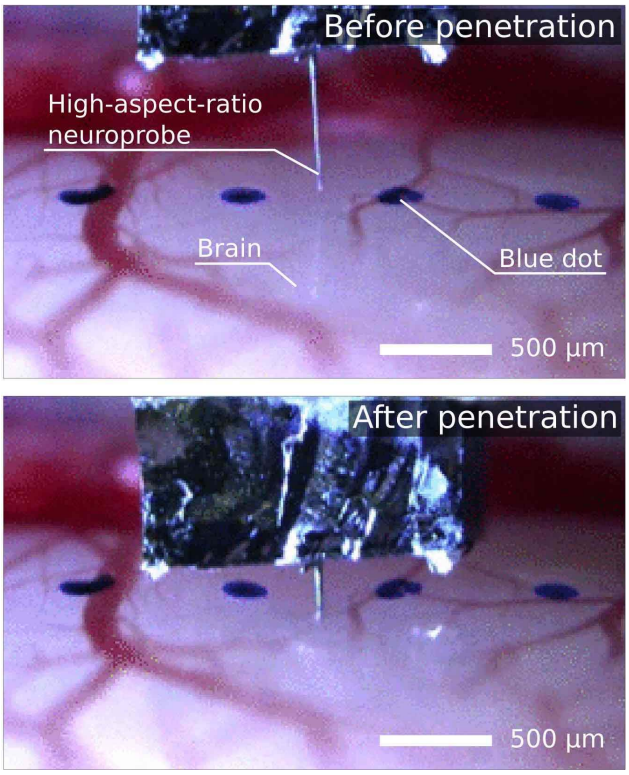

Figure 4: Photographs showing before (top) and after (bottom) a needle penetration into a mouse's brain (cerebral cortex, dura removed). The needle smoothly penetrated the brain without significant tissue deformation (blue dots used for analysis of the tissue deformation).

***In vivo* recording**

In vivo recordings were demonstrated using an anesthetized mouse. After removing the skin, skull, and dura matter, the packaged needle, which was connected to an amplifier system [SH16 head amplifier (input impedance: 10^{14} Ω), PZ2 preamplifier, RZ2 processor, Tucker Davis Technologies Inc.], was located over the mouse's brain and penetrated the somatosensory cortex (barrel field). The mouse's whiskers were fixed on a vibrator for the somatosensory stimulation. Figures 5a and 5b show schematic image of the experiment setup and photograph of the device placement, respectively. All experimental procedures were approved by the Committee for the Use of Animals at Toyohashi University of Technology, and all animal care followed the Standards Relation to the Care and Management of Experimental Animals (Notification No. 6, March 27, 1980 of the Prime Minister's Office of Japan).

During the sensory (whisker) stimulations, action potentials (spikes) with the amplitude of ~ 100 μV_{p-p} were observed. Note that the duration of the sensory stimulation was 6 s. The top panel in figure 6 shows the detected signals, which was filtered in the frequency band 500 Hz to 3 kHz, indicating that spike signals were clearly observed while the sensory stimulation. The middle and bottom panels in figure 6 show the peristimulus time histogram (PSTH) and raster plot diagram for the action potentials, respectively. The activity is in response to somatosensory stimulation with a latency of ~25 ms.

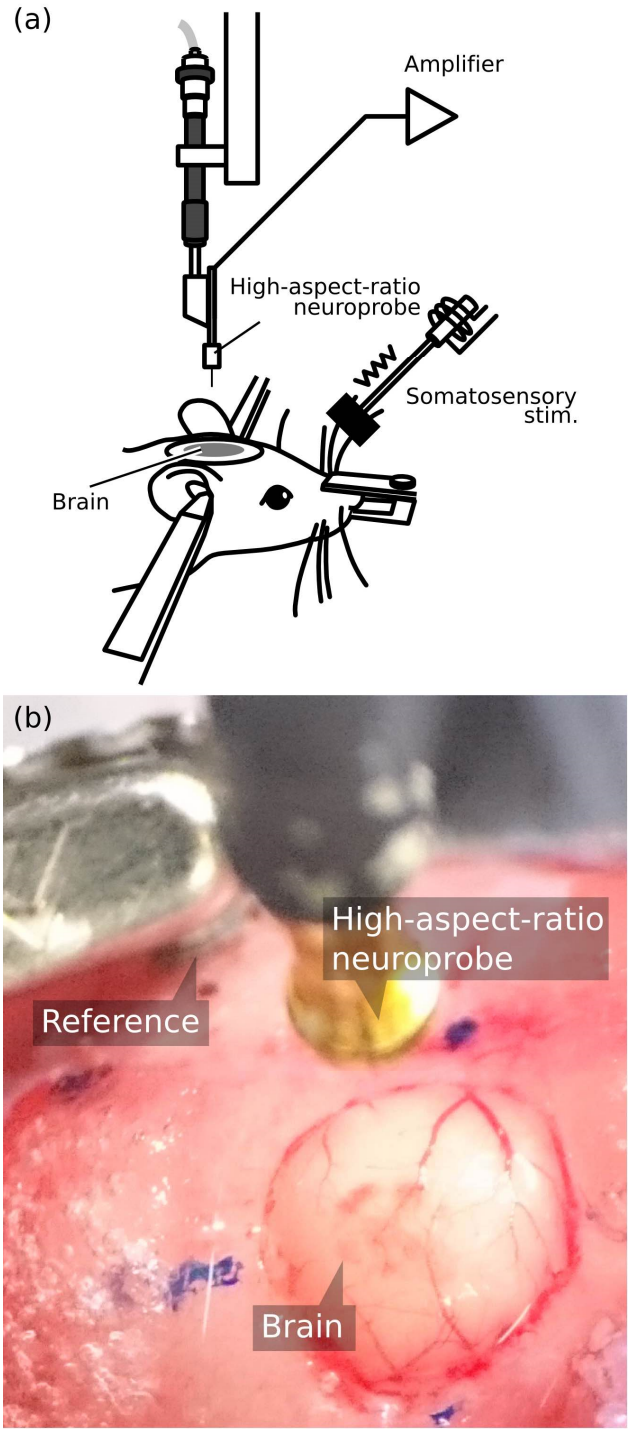

Figure 5: In vivo recording from the sensory cortex of a mouse. (a) Schematic image of the animal experiment. A packaged high-aspect-ratio needle electrode and a somatosensory stimulator (whisker vibrator) are connected to an amplifier/controller system. (b) Photograph showing needle device before the penetration.

DISCUSSION

We fabricated 5-µm-diameter and 400-µm-length ultra high-aspect-ratio needle-electrode for electrophysiological measurements. Because of the needle diameter, mechanical stresses of the brain tissue was minimized, as demonstrated in needle penetrations using a mouse's brain. Although the needle has such small electrode site, the impedance of the needle is low enough to detect spike signals from the brain by the tip modification with Pt black.

With VLS growth, we assembled the microneedle with the length of 400 µm, which was long enough to contact cell layers in the cerebral cortex. Our previous reports showed that the VLS needle with <210 µm in length successfully recorded neuronal spikes [2, 3]. Thus our current 400 µm needle would penetrate deeper layers than previous ones. This extension of ~200 µm helps examining layer specific function, and application to the other animals having thick cortex and the pia mater such as monkeys.

Longer needles (> 400 µm) with the same diameter might be formed by increasing the time of VLS growth (> 7 hours). Because of the insufficient needle's stiffness, such high-aspect-ratio needles might cause the needle's buckling due to the penetration pressure resulting in failure in penetration. The needle's stiffness can be enhanced by employing a scaffold at the needle base. We have proposed a biocompatible and dissolvable scaffold by using a silk film [6].

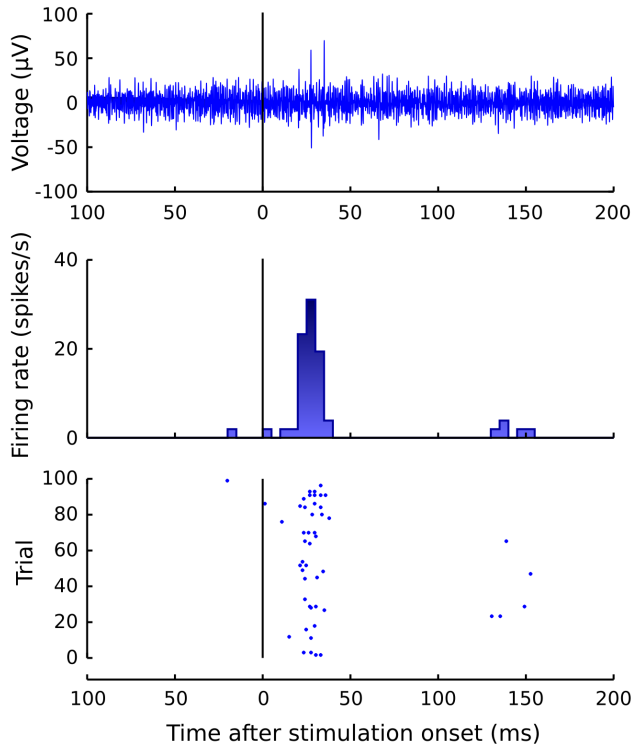

Figure 6: In vivo measurement results. Top, middle, and bottom graphs show detected signals (frequency band: 500 Hz - 3 kHz), histogram of firing rate (PSTH), and raster plots, respectively. Black line in the graph shows the onset of the somatosensory stimulation (0 s).

CONCLUSIONS

We reported 5-μm-diameter and 400-μm-length ultra high-aspect-ratio needle electrode for neuronal action potential recordings *in vivo*. Because of the tiny needle geometry (5 μm in diameter), the needle can smoothly penetrate a mouse's brain tissue *in vivo*, while the neuronal signals including action potentials were detected.

As a future work, the needle-electrode device will be implanted in a brain for chronic applications, including brain-machine interface (BMI) technology [7, 8]. Although we demonstrated *in vivo* recordings using a mouse's brain, the needle with the length of 400 μm is applicable to monkey's brain. Since the block module can further be minimized (< 1 × 1 mm^2, e.g. 200 μm^2) by stealth dicing, it is possible to fabricate the high density and 100% yield needle electrode arrays. Moreover, other functionalities, including intracellular recording nanoelectrode [9], microtube–based drug delivery [10], and optical waveguide [11], can be integrated with the high-aspect-ratio needle electrode array by using the same concept of block module.

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