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A Magnetically Assembled High-Aspect-Ratio Needle Electrode for Recording Neuronal Activity

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Microelectrode devices, which enable the detection of neuronal signals in brain tissues, have made significant contributions in the field of neuroscience and the brain–machine interfaces. To further develop such microelectrode devices, the following requirements must be met: i) a fine needle's diameter (<30 µm) to reduce damage to tissues; ii) a long needle (e.g., ≈1 mm for rodents and ≈2 mm for macaques); and iii) multiple electrodes to achieve high spatial recording (<100 µm in pitch). In order to meet these requirements, this study herein reports an assembly technique for high-aspect-ratio microneedles, which employs a magnet. The assembly is demonstrated, in which nickel wires of length 750 µm and diameter 25 µm are produced on a silicon substrate. The impedance magnitude of the assembled needle-like electrode measured at 1 kHz is 5.6 kΩ, exhibiting output and input signal amplitudes of 96.7% at 1 kHz. To confirm the recording capability of the fabricated device, neuronal signal recordings are performed using mouse cerebra in vivo. The packaged single microneedle electrode penetrates the barrel field in the primary somatosensory cortex of the mouse and enables the detection of evoked neuronal activity of both local field potentials and action potentials.

Microelectrodes, which penetrate the brain tissue and are located near neurons, enable the electrical recording of neuronal signals at a high spatiotemporal resolution. Such high spatiotemporal signal quality of the neuronal activity is necessary to understand neuronal networks and the working of brain.[1] Owing to the small size and high density of neurons in tissues, needle-like microelectrode devices are commonly used in neuronal recordings; such devices have made significant contributions in the fields of neuroscience and brain–machine interfaces.[2–7] To further develop such microelectrode devices, a microelectrode should i) have a fine needle diameter (<30 µm) to reduce damage to tissues. Several groups have demonstrated chronic recordings using >30 µm diameter wire-electrodes (e.g., four-month recordings of 50 µm diameter wires using rats[8] and five-year recordings of 30–50 µm diameter wire arrays using monkeys[9]). Further requirements for a microelectrode device include ii) a long needle (e.g., ≈1 mm for rodents[10,11] and ≈2 mm for macaque cortices[12]) and iii) multiple electrodes with a high density to achieve high spatial recordings (<100 µm in pitch).

To meet these requirements, we proposed an assembly technique for vertical microwires on a substrate using the magnetic force. **Figure 1**a1–a4 shows the principle of the proposed assembly. A magnetic wire is placed on a substrate that comprises a through hole [Figure 1a1]. The wire is then magnetized by applying a magnetic force [Figure 1a2]. The wire is magnetized as an n-pole; one end is repelled from the substrate and the other is attracted to the substrate [Figure 1a3]. After sliding the magnet under the through hole, the wire falls into the through hole [Figure 1a4]. This principle enables the magnetic assembly of microelectrodes for neuronal recording. Characteristics of both a fine electrode diameter and a long electrode are achieved by using microscale diameter wires and a photolithography-based etching process. In addition, these wires are assembled into through holes in the platform of the device substrate, allowing an electrical connection from the back side of the substrate [Figure 1b1].[13,14]

In this study, we report this magnetic assembly using a nickel (Ni) wire as the electrode and a silicon (Si) substrate as the device's platform. We used Ni wires with a 25 µm diameter

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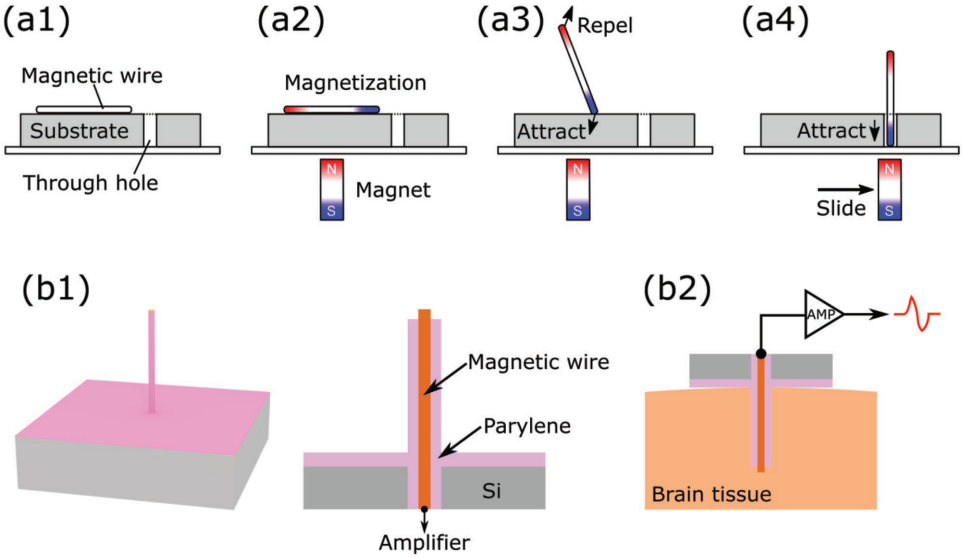

Figure 1. Magnetically assembled needle-microelectrode for neuronal recording. a1) Placement of magnetic wire on substrate. The substrate comprises through hole. a2) Magnetization of wire by applying magnetic force. a3) Wire standing. Wire's edge, which is magnetized as n-pole, repels from the substrate and the other is attracted to the substrate. a4) Falling wire into the through hole by sliding magnet. b1) A schematic and cross-sectional view of the device after parylene coating over the assembled magnetic wire. b2) A schematic showing the device used for neuronal recording.

and a 750–6500 µm length, which were assembled into 250 µm depth through holes in the Si substrate. The in vivo neuronal signal recording capability was demonstrated using a mouse brain [Figure 1b2].

Due to the fact that the length of a microwire with a desired diameter is determined using the photolithography-based cutting process, the proposed assembly technique allows the fabrication of needle electrode arrays with numerous wire diameters and lengths. Such methodology has not been realized using conventional approaches.

In order to prepare a Ni microwire with a defined length, we developed a wire cutting process. **Figure 2** shows the cutting process, in which several Ni wires are placed in parallel on a Si substrate and fixed with double-sided tape [Figure 2a1]. Herein, we used a Ni wire with a diameter of 25 µm. Subsequently, these wires were covered with a photoresist (AZ 5218) by spray coating [Figure 2a2]. The cutting lines in these wires were precisely defined by the photolithography process [Figure 2a3], and the wire was cut by etching the exposed wire sections with an aluminum etchant (H_3PO_4:HNO_3/CH_3COOH:H_2O = 10:1/1:2, at 60 °C) for 20 min [Figure 2a4; the photoresist was damaged (disconnected) due to the etching process]. The cut wires were thus obtained after removing the photoresist [Figure 2a5].

The microwires prepared via the cutting process were vertically assembled on a Si substrate magnetically. We used a 250 µm thick Si substrate with a 1.1 µm thick silicon dioxide layer, which had patterns of >30 µm diameter holes produced by photolithography and etching with a buffered hydrofluoric acid solution. With these oxide hole patterns as a hard mask, deep reactive ion etching (Deep-RIE) of the Si substrate formed >30 µm diameter and 250 µm deep through holes in the Si substrate [Figure 2b1]. For substrate insulation, parylene-C was coated at a thickness of 1 µm over the substrate, resulting in a parylene coating not only on the top and back of the substrate, but also on the sidewalls of the formed through holes [Figure 2b2]. After substrate preparation, ultraviolet (UV) tape was sealed onto the back of the substrate to prevent the wires from penetrating through the holes of the substrate [Figure 2b3].

Ni microwires, as prepared by the cutting process, were spread on the top surface of the substrate [Figure 2b3]. By applying a magnetic force (300 mT) using a permanent magnet, these wires stood vertically. These wires could be moved, following the magnet's position, so as to fall into the through holes and fix the position in the substrate [Figure 2b4]. Excess wires were similarly removed from the substrate surface using the permanent magnet. In order to fix the microwires to the substrate, the Si substrate was placed in a parylene coater and coated with a 5 µm thick parylene layer [Figure 2b5], with the magnet placed underneath the substrate during the coating. The parylene coating also results in the electrical insulation of the Ni wire. After parylene coating, the UV tape at the substrate's back was peeled off. Oxygen (O_2) plasma removed parylene at the wire's tip [Figure 2b6], resulting in an exposed Ni tip. Finally, the substrate was diced into 1 × 1 mm^2.

We demonstrated the proposed assembly technique with Ni wires and confirmed that this technique was capable of assembling both single needle and an array of needles of various lengths. **Figure 3**a1–a3 shows the assembly of single needles on the Si substrate using 25 µm diameter wires with lengths of 2500, 4500, and 6500 µm. The Si substrate had 30 µm diameter through holes of depth 250 µm. Thus, effective needle lengths were 2250, 4250, and 6250 µm, respectively. We also demonstrated the assembly of needle arrays. Figure 3b–d shows assembled single-needle, 2 × 2-needle, and 8 × 8-needle, respectively, in which needles were fixed to each substrate and electrically insulated with parylene. The length of each Ni wire used in the assembly was 750 µm (diameter = 25 µm). The pitch between the through holes in each Si substrate was 100 µm [Figure 3c,d].

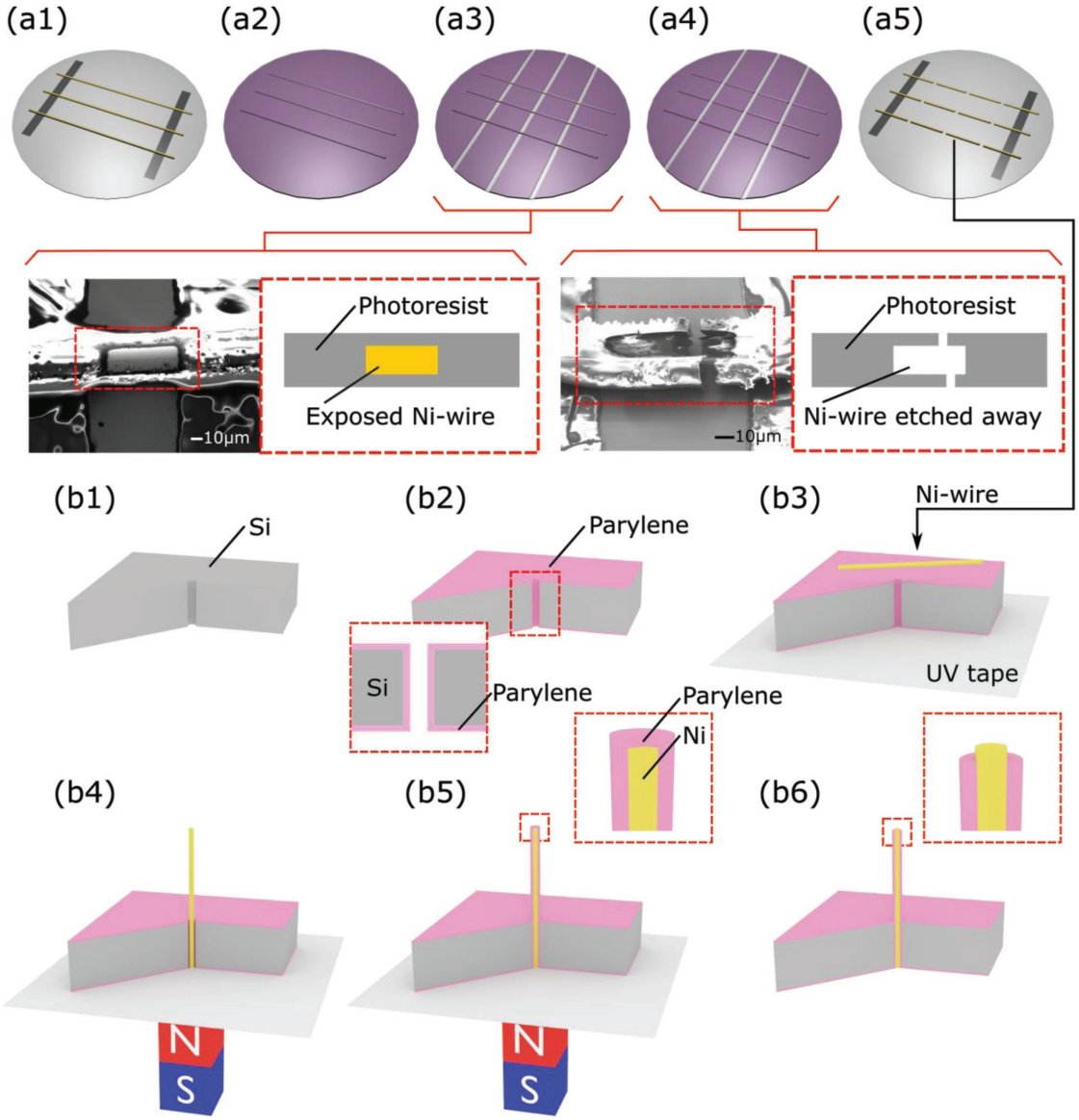

Figure 2. Process of the magnetic assembly of a Ni wire. a) Preparation of Ni wires. a1) Ni wires are fixed in parallel on a Si substrate with tape. a2) Ni wires are coated with a photoresist using spray coating, a3) and the cutting lines in these wires are defined by a photolithographic process. a4) The Ni wires are cut by etching the exposed wire sections away, a5) and the cut wires are obtained by removing the photoresist. b) Magnetic assembly of the cut Ni wire. b1) Si substrate with through holes with Deep-RIE. b2) Parylene coating of the Si substrate for substrate insulation. b3) Placement of the cut Ni wire (a) on the substrate. In order to prevent wires from penetrating through the substrate's through holes, the substrate has UV tape on the back. By applying a magnetic force, the wire stands up. b4) The Ni wire moves, following the magnet's position, and then falls into a through hole. b5) The assembled wire is coated with parylene, and b6) then the tip section is exposed.

As seen in the 8 × 8 array (100 μm pitch), some though holes were not filled with wires because the wires were trapped by previously assembled vertical wires during assembly. Another reason for this is that some wires were magnetized during the assembly and became attached to one another. The number of assembled wires was 28 (44% yield) [Figure 3d2]. We also demonstrated the 8 × 8 assembly using a pitch of 50 μm, confirming the reduction in the number of assembled Ni wires (19 wires, 30% yield). To achieve the assembly with a high efficiency, one method is to use automated assembly equipment,[14] which automatically moves either the substrate or magnet in plane with the optimized movement parameters.

A single-needle electrode was assembled on a Si block using the proposed technique. This was followed by the construction of the device package, electrical characterizations, and the animal experiment. **Figure 4**a shows photograph of the fabricated device die. The die size was 1 × 1 mm^2, which device geometry is applicable to a mouse brain in vivo.[15] The length and diameter of the assembled Ni wire were 750 μm (effective length = 500 μm) and 25 μm, respectively. The fabricated device die was packaged with a pin connector for electrical

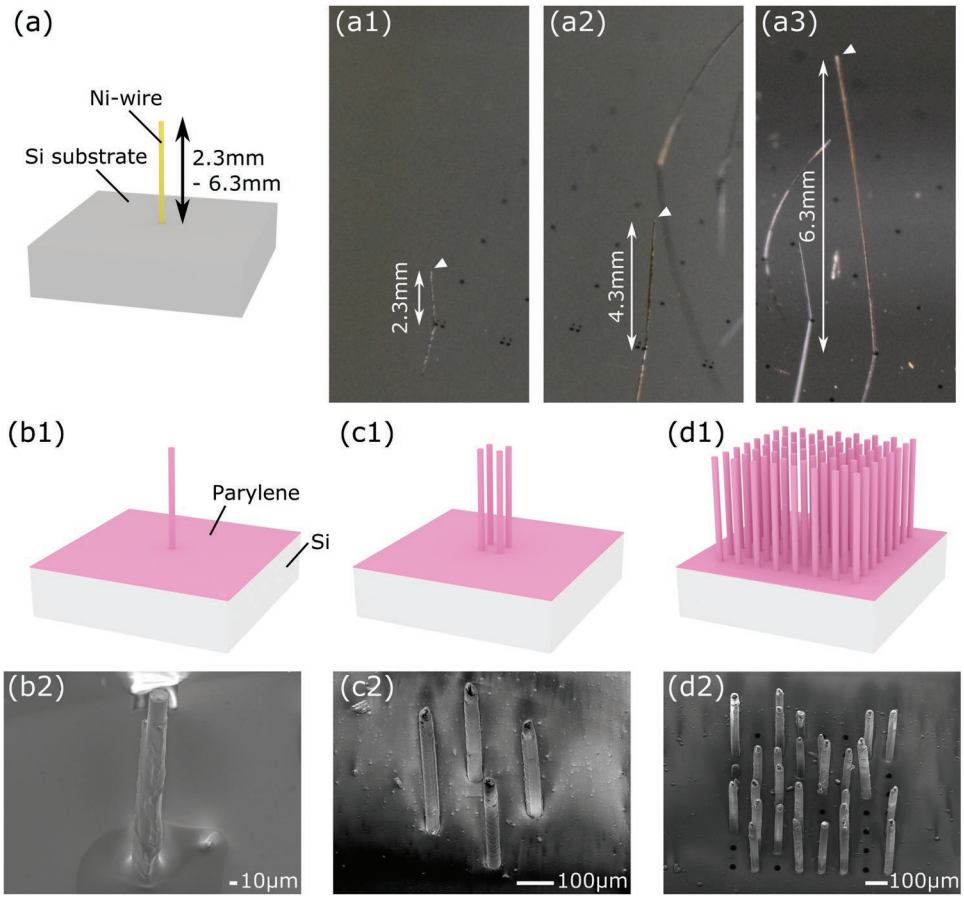

Figure 3. Magnetically assembled Ni wires. a) Assembly of needles with lengths of over 1 mm. Photographs show a1) 2.3 mm, a2) 4.3 mm, and a3) 6.3 mm length needles. b–d) Assembled 750 μm length needles. Schematics and scanning electron microscope (SEM) images show b1,b2) single, c1,c2) 2 × 2, and d1,d2) 8 × 8 needles. These wires are coated with parylene (thickness: 5 μm).

interconnection via the device's back [Figure 4b]. Conductive epoxy (CircuitWorks Conductive Epoxy; Chemtronics, Kennesaw, GA, USA) was used for the electrical connection between the back of the device and the pin connector. The sidewall of the Si die was covered with UV-cured resin as an insulator.

The electrode–electrolyte interfacial electrical impedance is an important characteristic of the electrode. The Ni wire assembled on the Si die was encapsulated with a 2 μm thick insulating layer of parylene, while the tip section was exposed to record the electrical signals of neuronal activity [Figure 4c]. The impedance characteristics of the pin-packaged needle electrode in a saline solution were measured [Figure 4d]. The impedance magnitude of the Ni-tipped needle measured in a saline solution at 1 kHz was 3.25 MΩ. This was reduced to 5.56 kΩ (1 kHz) after plating platinum black (Pt black) at the Ni tip section. We also measured the output/input (O/I) signal amplitude ratio of the electrode in a saline solution [Figure 4e]. The O/I ratios measured at 500–3000 Hz were 98.0% at 500 Hz, 96.7% at 1 kHz, and 92.5% at 3 kHz.

To confirm the recording capability of the fabricated device, neuronal signal recordings were taken for a mouse cerebral cortex in vivo. Under anesthesia (0.5% chlorprothixene solution 0.1 mL per 10 g and 10% urethane solution 0.05 mL per 10 g body weight), cranium and dura mater (0–3 mm caudal and 1–4 mm lateral from bregma) were surgically removed. The packaged device was inserted into the barrel field in the primary somatosensory cortex (S1B) with micromanipulator [**Figure 5**a]. Evoked potentials were recorded after needle penetration, during which the mouse's principal whiskers were physically stimulated using an electromagnetic vibrator driven by electrical pulses (2 ms in duration). Figure 5b shows the waveforms of local field potential (1–100 Hz, LFP), with an amplitude of 160 μV in 17 ms peak latency from stimulation onset. Figure 5c shows raw waveform (800–3000 Hz) acquired during a sample trial. Spike activities were detected by using a threshold of –20 μV (root-mean-square voltage noise = 3.91 μV). Figure 5d,e shows the raster plot for 100 trial stimulations and peristimulus time histograms (PSTHs) of the spike activities. First peak of firing rate (3971 spikes s^{-1}) appeared at 14 ms after stimulation onset. We could not uniquely isolate the single unit from the recorded spike signal by scrutiny based on the enlarged waveform (not shown).

In this paper, we used Ni wires with a diameter of 25 μm. Although we did not conduct any biosafety assessment experiments for such wires, several groups have demonstrated chronic recordings using >30 μm diameter wire-electrodes (e.g., 50 μm diameter[8] and 30–50 μm diameter wires[9]). Saxena and

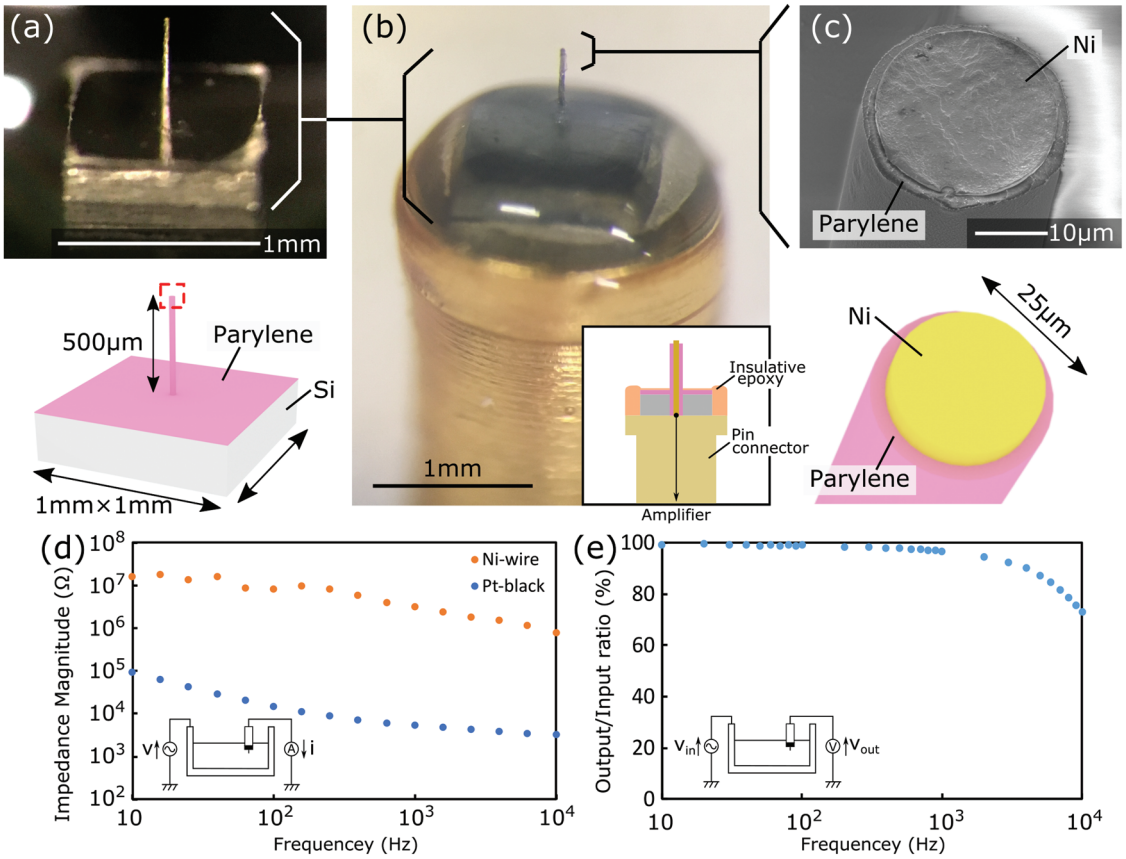

Figure 4. Device packaging and electrical characteristics of the Ni needle electrode. a) A photograph and schematic of the magnetically assembled needle electrode device. b) A photograph showing the needle electrode packaged with a pin connector. Herein, conductive epoxy was used for the electrical connection between the back of the device die and the pin connector. The sidewall of the Si substrate is covered with an insulating resin. c) A schematic and SEM image showing the tip section of the Ni wire. d) The impedance characteristics of the pin-packaged needle electrode measured in a saline solution. The graph shows data for both before and after Ni wire modification with Pt black. e) Output/input amplitude ratio of the pin-packaged needle electrode (Pt black) measured in a saline solution.

co-workers reported geometry-dependent-chronic blood–brain barrier (BBB) breaches for electrodes, indicating that a BBB breach in a four-month electrode-implanted rat was enhanced by a 50 μm thick "Michigan probe" compared to a 50 μm diameter wire.[8] It is known that a BBB breach activates the glia and astrocytes to form scars around hemorrhages.[16] Additionally, Saxena and co-workers explored the correlation of the extent of a BBB breach with the electrode function.[8] It is known that further minimizing an electrode to a diameter of <10 μm potentially reduces the recruitment of activated microglia and reacted astrocytes in the tissue.[17,18] Several groups have developed <10 μm diameter needle electrodes (e.g., a ≈7-μm-diameter electrode[19] or a ≈5-μm-diameter electrode[15,20]). To minimize the diameter of the needle, 10 μm diameter Ni wire can be fabricated by thinning a >10 μm Ni wire. Another method is to use a wire with a small diameter made of a nonmagnetic material that is subsequently coated with magnetic material for the magnetic assembly.

Here, we demonstrated a magnetic assembly of Ni wires with lengths <6 mm [Figure 3a1–a3]. Furthermore, to confirm the limitation of the length of the Ni wire in the proposed assembly technique, we used longer Ni wires with lengths of 10–30 mm. By applying the same magnetic force (300 mT), it was confirmed that Ni wires with lengths of 10 and 15 mm stood vertically [Figure S1a,b, Supporting Information], whereas wires longer than 20 mm did not stand up completely [Figure S1c,d, Supporting Information]. Because the magnetic force is proportional to $1/r^2$, where r is the distance from the magnet, the wire assembly with the proposed technique is limited by the length of the wire (<15 mm).

We demonstrated a vertical magnetic assembly of Ni microwires on a Si substrate. The proposed technique can be used for the assembly of not only Ni microwires but also other materials, including nonmagnetic wires, in which the wires are coated with a magnetic material (e.g., Ni, Co, or Fe). To further achieve a less invasive electrode, a tungsten (W) wire can be used as the core electrode. W wires (a nonmagnetic material) have a Young's modulus of 411 GPa, which is higher than that of Ni microwires (200 GPa) and conventional Si-microelectromechanical systems (MEMS) electrodes (185 GPa). Such a property allows for a reduction in the diameter and an increase in the length of needle. For example, to make W wires with a diameter of 5 μm and a length of 1000 μm stand vertically under a magnetic force of 300 mT using a permanent magnet (samarium–cobalt magnet), a Ni layer with a calculated thickness of more than 30 nm over the W wire is required. The proposed process is also applicable to

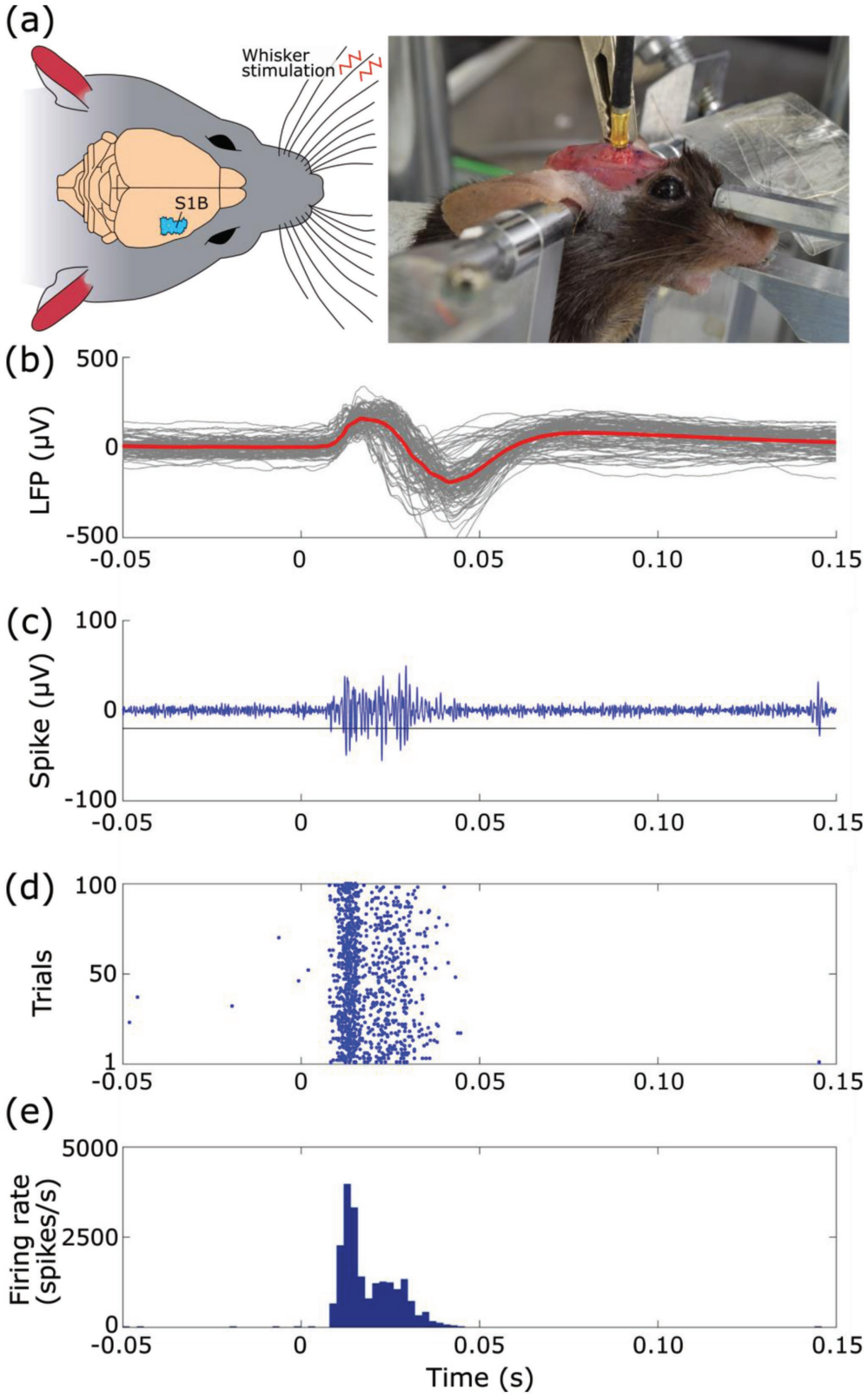

Figure 5. In vivo neuronal recording with the Ni needle electrode device. a) A schematic and photograph of in vivo recording using a mouse. The needle electrode penetrates the barrel field in the primary somatosensory cortex (S1B). The mouse's whiskers are physically stimulated during the recording. b) Wideband (1–100 Hz) and c) spike-shaped waveforms (800 Hz–3 kHz) derived from the needle electrode device [$n = 100$ trials for (b), single trial for (c)]. The average (red line) in (b) is taken from 100 trials (gray lines). d,e) Raster and PSTHs of the spike-shaped waveforms in the 100 trial stimulations.

glass fiber, which guides light and is promising for imaging purposes and optogenetic stimulation.[21–23]

Here, we have demonstrated in vivo neuronal signal recording from a mouse brain using a single-needle electrode that was packaged with a pin-type connector. The proposed technique enables the batch assembly of vertical Ni microwires on a Si substrate, as demonstrated in the assembly of arrays of 2 × 2 (100% yield) and 8 × 8 (yields of 30% for 50 μm in pitch and 44% for 100 μm in pitch) [Figure 3c,d]. In order to achieve an array of needle electrodes for multisite recording of neuronal activity, these needle electrodes should be electrically connected from the back of the Si substrate. One way to realize the electrode array is to use a wire bonding technique in which the metal bonding wire connects between the bottom of the needle electrode exposed from the Si substrate and the bonding pad of the package substrate. However, the wire bonding technique is limited by the number of needles and the pitch between needles. Using a selective electrical connection employing an anisotropic conductive paste or anisotropic conductive film between the needle bottoms and the contact pads of the package substrate might solve these issues and enable the development of a high-density electrode array device. Such a magnetic-assembly-based electrode array device will be reported in the future literature.

We hence demonstrated neuronal recording from a mouse's somatosensory cortex in vivo. This device offers additional opportunities for recording neuronal signals from numerous areas, including the visual and auditory cortices. The material used in conventional MEMS electrodes is Si, which introduces the issue of noise owing to the photoelectric effect during visual stimulation (e.g., light stimuli to the mouse's eyes). Due to the fact that the material of our electrode is Ni, the proposed device can eliminate the noise resulting from the photoelectric effect.

The proposed technique allowed the assembly of long needle electrodes with lengths of 500–6250 μm (Figure 3), thus enabling the possibility of recording neuronal activities in the deep brain. However, such long electrodes suffer from bending during tissue insertion, preventing electrode penetration. In order to achieve penetration for such long electrodes, we proposed the use of a dissolvable scaffold of silk.[24] This scaffold can support the needle temporarily but dissolves upon contact with the biological tissue (saline solution), resulting in long electrodes being able to penetrate into the tissue.

We demonstrated multiunit signal recordings from a mouse's somatosensory cortex using a 25 μm diameter needle electrode. To record single-unit signals, the diameter of each needle is an important parameter for the electrode device. The conventional electrode used in single-unit recording is a W electrode with a tip diameter of ≈10 μm. To increase the probability of unit recording, wires with diameters <30 μm are required. Candidate processes to achieve such a fine tip for a needle electrode (<25 μm) include plasma etching and electrolytic polishing.

In this paper, we proposed a magnetic assembly of Ni microwires in the through holes of a Si substrate, thus fabricating vertical microneedle electrodes with a fine diameter, long structure, and backside electrical connection. We demonstrated the assembly of arrays of needle electrodes with a diameter of 25 μm and lengths ranging from 500 to 6250 μm. The assembled single-needle device showed neuronal recording capability, as demonstrated in in vivo recordings using the somatosensory cortex of a mouse. Compared with conventional methodologies, such as Si-MEMS electrodes, the proposed methodology enables the fabrication of an array device of fine diameter and long electrodes with a backside electrical connection. Although the electrical recording capability of the device has been demonstrated, this methodology is applicable to the fabrication of numerous devices with other functionalities, including optical waveguiding fibers for optogenetic applications and hollow microtubes for drug delivery. The proposed assembly technique is applicable to not only Si substrates, but also Si-complementary metal-oxide-semiconductor (CMOS) and other flexible and stretchable substrates.

Experimental Section

All experimental procedures using animals were approved by the committees 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).

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

assembly, magnets, needle electrodes, neural recording

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