Long-term in-vivo recording performance of flexible penetrating microelectrode arrays

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Abstract

Objective. Neural interfaces are an essential tool to enable the human body to directly communicate with machines such as computers or prosthetic robotic arms. Since invasive electrodes can be located closer to target neurons, they have advantages such as precision in stimulation and high signal-to-noise ratio (SNR) in recording, while they often exhibit unstable performance in long-term in-vivo implantation because of the tissue damage caused by the electrodes insertion. In the present study, we investigated the electrical functionality of flexible penetrating microelectrode arrays (FPMAs) up to 3 months in in-vivo conditions. Approach. The in-vivo experiment was performed by implanting FPMAs in five rats. The in-vivo impedance as well as the action potential (AP) amplitude and SNR were analyzed over weeks. Additionally, APs were tracked over time to investigate the possibility of single neuron recording. Main results. It was observed that the FPMAs exhibited dramatic increases in impedance for the first 4 weeks after implantation, accompanied by decreases in AP amplitude. However, the increase/decrease in AP amplitude was always accompanied by the increase/decrease in background noise, resulting in quite consistently maintained SNRs. After 4 weeks of implantation, we observed two distinctive issues regarding long-term implantation, each caused by chronic tissue responses or by the delamination of insulation layer. The results demonstrate that the FPMAs successfully recorded neuronal signals up to 12 weeks, with very stably maintained SNRs, reduced by only 16.1% on average compared to the first recordings, although biological tissue reactions or physical degradation of the FPMA were present. Significance. The fabricated FPMAs successfully recorded intracortical signals for 3 months. The SNR was maintained up to 3 months and the chronic function of FPMA was comparable with other silicon based implantable electrodes.

1. Introduction

Neural interfaces are an essential tool to enable the human body to directly communicate with machines such as computers or prosthetic robotic arms, and to restore the senses lost by diseases or accidents. A variety of neural electrodes have been developed and implanted in the brain for the purposes of controlling robotic arms by analyzing brain signals [1–5], restoring the vision [6–10] and the somatosensory function [11, 12], and delivering auditory information [13, 14]. These electrodes have been used to acquire neural signals from or transmit stimulating signals to neurons.

Since invasive electrodes can be located closer to target neurons, they can stimulate neurons with
higher selectivity and detect neural signals with higher precision and signal-to-noise ratio (SNR) than non-invasive counterparts [15]. However, invasive electrodes often exhibit unstable performance in long-term in-vivo implantation because of the tissue damage caused by the electrodes insertion and the failure of implants. First, penetrating electrodes such as deep brain stimulation electrodes and intra-cortical electrodes damage the brain inevitably during implantation, which causes immune responses by bleeding, blood vessel damage, and neural cell deaths [16, 17]. In addition, foreign body reaction or neuronal changes near the sheath layer consisting of microglial cells and astrocytes can be induced by insertion wound healing [18–20]. Consequently, the glial scar forms around the electrodes and pushes neurons away from the active sites of electrodes, resulting in the deterioration of electrodes’ recording or stimulating function [21]. At the same time, the degradation of implanted devices can occur during implantation over a long time [22].

Previously, a flexible penetrating microelectrode array (FPMA) was developed [23] and demonstrated the capability in neural recording from the peripheral nerve up to 4 weeks [24]. In the present study, we investigated the electrical functionality of the FPMA during longer-term implantation up to three months in the in-vivo brain. First, we examined the in-vivo electrode impedance, which is a typical measure to indicate the characteristics of electrodes and their surroundings, based on preceding studies [25–27]. We performed the neural signal acquisition over 3 months to analyze the long-term characteristics of implanted electrodes. From the acquired neuronal signals, electrical parameters such as the level of background noise, the amplitude of action potentials (APs) and SNR were analyzed. Lastly, we examined the explanted devices and the brain tissues immunohistochemically by staining nuclei, microglia, and astrocytes.

2. Methods

2.1. Integrated flexible penetrating microelectrode array (FPMA) device

The integrated FPMA device was comprised of three parts: an FPMA to record neuronal signals, an interconnection cable to connect the FPMA with an amplifier/data acquisition system, and a pin header, as shown in figure 1. First, the FPMA, a microneedle array with a flexible base made of polydimethylsiloxane (PDMS), was fabricated as described in a previous study [23]. First, Ti/Au in thicknesses of 50 nm/200 nm, which served as contact pads for electrical connection, were sputtered (SRN-110, Sorona Inc., Anseong, Korea) and patterned on a highly boron-doped, 1.30 mm thick silicon wafer. The wafer was etched in a grid pattern by deep reactive ion etching (RIE) (LPX PEGASUS, SPTS Technologies Ltd, Newport, UK), to generate 200 µm-deep trenches. The trenches were filled with PDMS and cured at 60 °C for 2 h, to create the flexible base of the FPMA. Next, the opposite side of the wafer was diced to generate columns that would become conical needle shapes by etching using HNA solution. The fabricated microneedles were designed to be 1100 µm in height and approximately 75 µm in diameter at the bottom, with a pitch between electrodes of 550 µm, as shown in figures 1(a) and (b). For in-vivo experiments, the FPMA were cut in an array of 4 × 4 electrodes, with dimensions of 2.2 mm × 2.2 mm and the PDMS base thickness of 200 µm. The electrode tips of FPMA were coated with Ti/Pt in thicknesses of 50/200 nm by using a sputter deposition system (DKSPT10-05-EIB, Daeki Hi-tech Co., Daejeon, Korea). To insulate the electrode array except the active electrode sites, 3 µm thick parylene-C was deposited by a low-pressure chemical vapor deposition (LPCVD) process using a parylene coating system (NRPC-500, Nuritech, Goyang, Korea), resulting in the tip exposure length of approximately 50 µm.

The second component, the interconnection cable, consisted of a flexible parylene-C cable and a flexible printed circuit board (FPCB) (figure 1(c)). The parylene-C cable was designed to be 48.4 mm in length, 3.4 mm in width at the FPMA site and 10.5 mm in width at the bonding site with the FPCB. All fabrication processes were performed at temperatures lower than 125 °C to prevent thermal oxidative degradation of parylene-C [28, 29]. To fabricate the flexible parylene-C cable, Ti in a thickness of 200 nm was deposited on a Si wafer as a sacrificial layer by using sputter deposition. The bottom layer of 6 µm thick parylene-C was deposited in the LPCVD chamber. The contact pads and conductive lines made of Cr/Au, in thicknesses of 50 nm/200 nm, were patterned by using lithography and wet etching processes. Next, the top insulating layer of 6 µm thick parylene-C was deposited. To expose the contact pads, Ti in a thickness of 200 nm was sputtered on the top insulating layer and patterned as a mask. RIE was used to expose the contact pads (VITA, FEMTO Science, Hwaseong, Korea). The used RIE conditions were 50 W radio frequency (RF)-biased source and 30 sccm of oxygen (O2). The flexible cable was released from the wafer by immersing it in Ti etchant (hydrofluoric acid solution) until it was floated. To connect the interconnection cable with the signal acquisition equipment, a custom-designed intermediate FPCB was used to avoid direct soldering on the parylene-C cable, which would cause the film cracking by oxidation of parylene-C under high temperature circumstances. The FPCB was fabricated based on polyimide, with a length of 10.3 mm and a width of 16.4 mm at the pin header site.

The FPMA was assembled with the interconnection cable and a pin header (850-10-050-10-00100, Mill-Max Mfg. Corp., New York City, NY, USA).
The FPMA was first connected with the parylene-C cable, which was in turn connected with the FPCB by using conductive epoxy. To protect the connected junctions, liquid PDMS was filled in the gap between the FPMA and the interconnection cable by capillary force. Subsequently, conductive epoxy and PDMS were cured at room temperature (25 °C) for 12 h. Finally, the 16-pin header was soldered on the FPCB, as shown in figure 1(c).

2.2. Surgery for FPMA implantation
FPMA implantation and data recording were performed with protocols approved by the Institutional Animal Care and Use Committee at Kyungpook National University. Five male Sprague-Dawley rats weighing between 290 and 540 g were used. Animals were anesthetized by a 2 ml intraperitoneal injection of a mixture of ketamine (36.3 mg ml⁻¹) and xylazine (1.8 mg ml⁻¹). Animals were then fixed in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, USA). The scalp was incised and spread for a clear vision of the implant site after subcutaneous injection of 2% lidocaine. The periosteal tissue was removed and the surface of the skull was cleaned with 3% acetic acid. Holes were carefully drilled and ten stainless steel screws (1 mm diameter) were implanted into the skull to anchor the shielding cap with dental resin. Two screws over the cerebellar cortex served as the ground and reference electrodes, while other screws were used as the anchor preventing the detachment of dental resin. A piece of skull in a size of 5 mm × 5 mm, which center was at +3.0 mm lateral to the bregma, was removed using a high-speed drill. Additionally, the dura mater was removed carefully. The FPMA was inserted into the somatosensory cortex by using a pneumatic inserter (Blackrock Microsystems, Salt Lake City, UT, USA). The implantation area was selected as a region that had sufficient spatial margins to implant the FPMA and to fix the shielding cap. The piece of the removed skull was put on the implanted FPMA to avoid the direct contact of the FPMA with dental resin. The dental resin lightly covered the skull to prevent the macro movement of the FPMA. Finally, a 3D-printed shielding cap was placed on the cured resin to protect the integrated FPMA device. The shielding cap was devised to minimize the behavioral influences of the animals, such as scratching the wound, on the implanted electrodes while the animals were returned to their home cage. Additional dental resin was used to fix the shielding cap with the skull. The schematic of the experimental setup and the photographs of the implanted animals are shown in figure 2.

2.3. Neural signal acquisition
In-vivo recording was conducted once a week after implantation for 12 weeks. In every recording session, the rat was anesthetized with a mixture of ketamine and xylazine to minimize the artifacts caused by movements. The dose of the drug and the condition of animals were strictly observed to maintain a stable experimental condition. The animal was placed in a custom-made Faraday cage to minimize background noises and interferences from powerline or electronic equipment. Intan RHD2000 data acquisition system (Intan Technologies, Los Angeles, CA, USA) was used to acquire in-vivo impedance at 1 kHz and to record neuronal activities. The signals were recorded at a
Figure 2. Schematic illustration of the recording setup. The FPMA was implanted at the somatosensory cortex (left in the red box). Two of the screws were used as the ground and reference electrodes, while the others were used to help fix the shielding cap, which was anchored on the skull through dental resin and screws to protect the implanted FPMA (middle in the red box). Signals were acquired while the animal was anesthetized (right in the red box).

2.4. Spike detection and analysis

First, pre-processing was conducted to remove background noises. Recorded raw data contained neuronal signals, power line noises, artifacts due to muscular motion, vessel contraction, and breathing noise [30]. Mechanical noises such as breathing or vessel contraction were mainly comprised of low frequency components under 300 Hz [31]. To obtain APs, the filtering process was essential to remove noises and LFPs under 100 Hz from the recorded raw data.

A commercial software, SPIKE 2 (CED, Cambridge, UK), was used to remove noises and sort out APs. A band-pass filter from 500 to 5000 Hz was used to remove background noises [25, 26, 32]. Spike detection and sorting processes were then conducted by using the threshold and shape-based sorting methods embedded in the software, which classified detected spikes into templates based on repeated AP shapes. The threshold value was set to be three times the root mean square (RMS) value of the pre-processed signal, which is conventionally used for threshold sorting [33]. Single spikes were identified when the waveform crossed the threshold and were centered in a 1.5 ms waveform snippet. The software compared all discovered spike waveforms and created a template for those if there were more than 20 spikes of similar waveform. To assess the electrical performance of the FPMA, we analyzed the raw data by using three parameters, i.e. the amplitude of APs, the RMS value of noise and the SNR. Sorted APs, detected by each electrode, were used to extract the average peak-to-peak amplitude of the spikes by that electrode. Signals other than spikes were considered as noise. The SNR was calculated by using the following equation [34, 35]:

$$\text{SNR} = \frac{\text{average of APs amplitude}}{2 \times \text{standard deviation of noise}}.$$  

The standard deviation of noise was the same as the RMS value of noise, because the mean value of noise was nearly zero.

The sorted APs from each single electrode was analyzed to track single neurons over 12 weeks. All sorted APs were normalized for a fair comparison between different APs. We compared the normalized amplitudes of first negative peak, first positive peak, and second negative peak with the time point of each peak over weeks. In addition, we conducted principle
component analysis (PCA) and calculated autocorrelation coefficients to examine the similarity of APs recorded at different weeks. The PCA was conducted using SPIKE 2 software. Also, the autocorrelation coefficient \( r \) between two signals \( Y_1(t) \) and \( Y_2(t) \) was calculated based on the following equation using MATLAB (MathWorks, Natick, MA, USA):

\[
\text{Corr}(Y_1, Y_2) = \frac{\int_{T_1}^{T_2} (Y_1(t) - \bar{Y}_1)(Y_2(t) - \bar{Y}_2) dt}{\sqrt{\int_{T_1}^{T_2} (Y_1(t) - \bar{Y}_1)^2 dt \int_{T_1}^{T_2} (Y_2(t) - \bar{Y}_2)^2 dt}}
\]

where \( T_1 \) and \( T_2 \) indicate the starting and ending time points of the recorded APs, and \( \bar{Y}_i \) represents the average of \( Y_i(t) \) \([36, 37]\).

2.5. Immunohistochemistry

Rats were intracardially perfused with 4\% paraformaldehyde. The brains were dissected out and post-fixed overnight at 4°C, and afterwards immersed in 30\% sucrose in phosphate-buffered saline (PBS) solution and dehydrated for 3 d at 4°C. The brains were embedded in optimal cutting temperature compound (Scigen, Paramount, CA, USA) and frozen at ~80°C. The 20 \(\mu\)m thick coronal sections were obtained using a cryostat (CM3050 S, Leica, Wetzlar, Germany). The prepared slices were labeled with the following primary antibodies: mouse anti-glial fibrillary acidic protein (GFAP) (3670 S, 1:200, Cell Signaling, Danvers, MA, USA) and rabbit anti-Iba1 (019-19741, 1:1000, Wako, Madison, WI, USA). The following fluorophore-conjugated secondary antibodies were used: Alexa 488 donkey anti-rabbit (711-545-152, 1:1000, Jackson Immuno Research Laboratories, West Grove, PA, USA) and Cy3 donkey anti-mouse (715-165-150, 1:1000, Jackson Immuno Research Laboratories, West Grove, PA, USA). The slices were counterstained with 4′6′-diamidino-2-phenylindole (DAPI) and images were taken using a confocal microscope system (C2+, Nikon, Tokyo, Japan). To analyze the population of astrocytes, nuclei, and microglia, the fluorescence intensity was measured at different distances from the centers of five electrodes, from 25 to 200 \(\mu\)m at a 5 \(\mu\)m interval [38]. The measured fluorescence intensity was normalized based on the intensity obtained at the area between 240 and 250 \(\mu\)m from the electrode center to visualize the spatial distribution of each cell type.

3. Results and discussion

3.1. Fabricated FPMA device

The integrated FPMA device, with 16 electrodes, is shown in figure 1(c). The overall dimensions of the FPMA were 2.2 mm \(\times\) 2.2 mm \(\times\) 1.3 mm, including microneedles with a height of 1.1 mm. Based on the scanning electron microscopic (SEM) images as shown in figures 1(a) and (b), the length and geometrical surface area of active electrode tips were estimated to be 56.3 ± 12.8 \(\mu\)m and 2136 ± 647 \(\mu\)m\(^2\), respectively. The total length of the integrated FPMA device was approximately 6 cm, including the flexible parylene-C cable and the FPCB, as shown in figure 1(c).

3.2. Recording of neuronal signals

Neural signals were monitored for 12 weeks to assess the long-term electrical performance of the FPMA. A representative example of recorded signals from a rat (rat 3) is shown in figure 3, which was recorded at 1 week after implantation. The 87.5\% of the electrodes could record the neuronal activities from the somatosensory cortex, except channels 3 and 15. To monitor the single-unit recording capability of the FPMA, we tracked the waveforms of APs detected by individual electrodes. The identical waveforms of APs could be observed continuously over weeks by the same electrode, as shown in figure 4(a). The amplitude of APs changed over time, but the same waveform was maintained, confirming the capability of same neuron recording over weeks. The autocorrelation in figure 4(b) shows the percentage of correlation coefficient in each recording session, indicating the similarities between APs. The correlation coefficient close to 1 indicated same spike shapes. In previous studies \([36, 39]\), signals obtained from the same neuron exhibited a similarity score of 81\% with correlation coefficients \( r \) values over 0.95, while signals obtained from different neurons showed a similarity score of 37\%. In the present study, the \( r \) value greater than 0.95 were 80\% in rat 4 and 85\% in rat 5, which implies that the recorded signals were obtained from identical neurons for 12 weeks. Also, AP waveforms detected over weeks were not significantly different through PCA analysis, as shown in figure 4(c). Through the two verification methods, it was confirmed that the APs shared common features over 12 weeks, indicating that they were generated by the same neurons.

3.3. Quantitative analysis of long-term electrical performance

The interfacial impedance between the electrode and the tissue, the peak-to-peak amplitude of APs, the RMS value of noise, and the SNR were analyzed to assess the electrical performance of the FPMA over 12 weeks. For instance, an increase in impedance would indicate that the encapsulation sheath composed of intermediate cells such as astrocytes and microglia is formed between the working electrodes and the reference electrode \([40–42]\). On the other hand, the delamination of insulation layer caused by water permeation may induce a decrease in impedance \([22]\). Also, the changes in APs amplitude and SNR indicate the change
Figure 3. Recorded neural signals obtained from the somatosensory cortex of rat 3 at 7 d after implantation. 87.5% of the electrodes except electrodes 3 and 15 could record APs.

in neuronal density in the tissue around the electrodes [38].

3.3.1. In-vivo impedance
The in-vivo impedance was measured at 1 kHz weekly to monitor the environmental changes at the implant site. Since the initial in-vivo impedance varied a lot according to the distribution of cells in the animal and the degree of opening of the electrodes, the relative change in impedance was more meaningful to indicate the environmental changes over time. Therefore, the impedance of each electrode at each week was normalized by the first-week impedance. Figure 5 shows the temporal changes in averaged relative impedance of all functional electrodes that could record neural signals at the first recording (For the criterion to decide functional electrodes, see section 3.3.2). Temporal changes in impedance of all electrodes including both functional and non-functional electrodes are shown in supplementary figure S1 available online at stacks.iop.org/JNE/18/066018/mmedia. The numbers of functional electrodes in rats 1–5 were 4, 9, 14, 11, and 13, respectively. The averaged impedance of functional electrodes at 1 week after implantation was 645, 125, 716, 812, and 1004 kΩ in rats 1–5, respectively. Before implantation, the impedance of functional electrodes was measured in PBS solution, resulting in 10.80 ± 1.41, 20.31 ± 6.61, 498.30 ± 268.85, 352.37 ± 246.91, and 745.62 ± 542.61 kΩ in rats 1–5, respectively, indicating that the exposed area of the FPMAs implanted in rats 1 and 2 was larger than that of other FPMAs. Generally, the impedance of all functional electrodes in the used FPMAs increased after implantation, suggesting the difference between PBS and an in-vivo brain. Meanwhile, the averaged impedances of non-functional electrodes were considerably higher than those of functional electrodes in most animals (except rat 4) from week 1, as shown in supplementary figure S1, indicating broken interconnections to the electrodes during the surgery.

In rat 2, it was exceptionally observed that the shielding cap was loosened by the movements of the rat, which resulted in a dramatic increase of impedance up to week 7 and a decrease afterwards (supplementary figure S2). In figure 5, the relative impedance in rat 1 decreased by 14% on average in the last week of recording compared to the first recording. One of the reasons of decreasing impedance was speculated to be the damaged surface of the insulation layer of the electrodes. As shown in figure 6(b), the coating material was delaminated from silicon microelectrodes, compared to the intact insulation as fabricated in figure 6(a). It is speculated that the water diffusion through coated parylene-C and weak adhesion between parylene-C and sputtered metal underneath could cause the delamination of parylene-C [43]. On the other hand, figure 6(c) shows that the insulation layer was tightly attached to the microelectrode explanted from rat 4, confirming that no delamination of insulation
layer occurred. In cases of rats 3–5, the impedance increased rapidly up to 4 weeks, which was speculated to be caused by biological responses that microglia and astrocytes encapsulated the implanted electrodes [17, 44, 45]. After the first 4 weeks, the relative impedance in rat 3 was gradually decreased by 29% at week 12 compared to week 4, with a fluctuation from week 8 to week 10. The relative impedance in rat 4 after week 4 remained consistently up until the animal was sacrificed, with only a small decrease of 14% at week 12 compared to week 4. In rat 5, the relative impedance increased steadily even after week 4, by 20% at week 12 compared to week 5.

3.3.2. AP amplitude, noise level and SNR
We determined the success in recording of each electrode, by calculating the SNR: electrodes with an

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**Figure 4.** Sorted spike waveforms from single neurons tracked by single electrodes. (a) Spike waveforms were obtained from a single neuron in rat 4 and rat 5 for 12 weeks. (b) The cross-correlation coefficients of rat 4 and rat 5 were analyzed with sorted spikes from week 1 to week 12. (c) The PCA result of sorted spikes at each week was overlapped to verify the similarity of spikes.
SNR lower than 2 were defined as non-properly-functioning electrodes and thus were not considered for further analysis. Based on this criterion, the ratio of the electrically functional electrodes in each array is shown in figure 7(a). Recording quality was evaluated by extracting the parameters such as the peak-to-peak amplitude of APs, background noise, and SNR, as shown in figures 7(b)–(d).

In the first recordings, the numbers of functional electrodes that were capable of measuring neuronal signals were 4, 9, 14, 11, and 13 in rats 1–5, respectively, which corresponded to 25%, 56.25%, 87.5%, 68.75%, and 81.25%. Only these functional electrodes were used in calculation of the amplitudes of AP in each rat. The averaged AP amplitudes were 43.44, 61.28, 152.86, 65.79 and 130.12 µV from rats 1–5, respectively. The RMS values of noise were 4.86, 6.64, 16.31, 6.89 and 12.61 µV, resulting in SNRs of 4.43, 4.45, 4.67, 4.79, and 5.16 from rats 1–5, respectively. For more details, see supplementary information, table S1.

In some FPMAs, AP recordings did not last longer than 6 or 7 weeks due to unstable environmental circumstances. For rat 2, even in an unstable circumstance that the fixation of the shielding cap was loosened, the signal recording was possible up to 7 weeks (see supplementary figure S3). For rat 3, the AP amplitude decreased dramatically during the first 4 weeks due to acute inflammatory reactions and was stabilized afterward. However, from week 7, the analysis of electrical parameters was not possible because the signals recorded by all electrodes exhibited SNRs lower than 2. We observed that the FPMA extracted from rat 3 was attached to dental resin, which was speculated to be the reason for the poor signal quality after week 7. On the other hand, rats 1, 4 and 5 did not exhibit any adverse effects or issues critical to recordings.

The averaged AP amplitudes from the last recordings in rats 1–5 were 33.97, 35.22, 95.47, 57.82 and 69.83 µV, with the SNRs of 3.75, 3.81, 4.58, 4.18 and 4.17, respectively, as shown in table S1 (supplementary information). Overall, the SNR of all analyzed electrodes was 4.70 ± 0.30 at the first recording and 4.10 ± 0.34 at the last recording. The SNR of three rats (rats 1, 4 and 5), with which APs were successfully acquired for 12 weeks, decreased only by 15.7 ± 3.2% at the end of the experiment, compared to the initially obtained values. From figures 7(b)–(d), the decrease/increase in AP amplitude was always accompanied by the decrease/increase in background noise. As the result, the SNR barely changed over the entire period of experiment. As for the electrodes implanted in rats 2 and 3, successful recordings were only possible up to 6–7 weeks, due to clear incidents such as loosening of the shielding cap or attachment of the FPMA to dental resin. On the other hand, in rats 1, 4 and 5 where successful recordings were possible for 12 weeks, the number of functional electrodes decreased only slightly from no reduction up to by 15.4% after 12 weeks.

In previous studies using the Utah electrode array (UEA), it was reported that the number of functional electrodes decreased by 18%–27% over 12 weeks [46, 47]. We also observed that electrically functional electrodes, which successfully recorded at the first recording, were consistently operating over the entire experimental period.

3.3.3. Summary of long-term electrical performance
From previous studies in literature, the UEA is a good example of comparison for the FPMA, with a key difference that the FPMA is fabricated based on a flexible PDMS base while the UEA is fabricated entirely based on rigid materials such as silicon and glass. Table 1 summarizes the long-term electrical functionalities of the FPMA and compares them with the UEA. The long-term functionalities of the UEA in in-vivo conditions were reported previously by multiple research groups [46–49]. In previous studies, SNR reductions with the UEA were reported to be from 33.6% to 37.3% after 24–30 weeks of implantation, while the SNR reduction with the FPMA was 12.7% after 12 weeks. For a fair comparison at 12 weeks, the SNR reduction with the UEA was reported to be 32% after 12 weeks. For a fair comparison at 12 weeks after implantation, SNR reductions with the UEA were about 21% in [46] and 36% in [47]. The impedance of the UEA was reported to increase up to 665% in [47, 48] but decrease by 85.4%–93.2% in other studies [46, 49] although the experimental periods were all different. In [46], Black et al reported the delamination of insulation layer after 20 weeks of implantation, accompanied by decreasing impedance. The decrease in ratio of functional electrodes in the UEA was reported to be from 26.4% to 82.7%, while it was 12.1%
in our present study. Overall, the change in number of functional electrodes, the change in impedance, and the change in SNR of the FPMA were comparable with those of the previously studied UEAs, suggesting that the FPMA can be used for a chronic experiment.

Based on the results in figures 5 and 7, the increase in impedance was generally accompanied by the decrease in AP amplitude. However, as the AP amplitude and noise level followed the same tendency, the change in SNR was much less significant. After 4 weeks, the impedance was observed to be either increasing or decreasing as shown in figure 5. In some case (e.g. rat 5), gradually increasing impedance along with decreasing AP amplitude was observed after week 4, due to chronic tissue responses such as foreign body granuloma, astrocyte ensheathing reaction, the formation of activated microglia around implanted electrodes, and neuronal degeneration [19, 38, 40, 50]. However, in other case (e.g. rat 1), decreasing impedance was observed to be accompanied by gradually decreasing AP amplitude after 4 weeks, with which the insulation layer was speculated to be delaminated. The reduction of SNR from all functional electrodes was only 15.7% on average after 12 weeks. Therefore, it can be concluded that the FPMA demonstrated reasonable signal quality for 12 weeks. It may be possible to acquire better quality signals by delivering anti-inflammatory drugs to the implant site [51–53], or by enhancing the adhesion of parylene-C insulation layer with the electrodes to prevent the delamination. The delamination
between parylene-C and electrode metal would be prevented by additional fabrication steps such as thermal annealing of parylene-C, applying adhesion promoter, or adding an interposed layer between metal and parylene-C [54–56]. In further study, we will attempt such additional processes to enhance the adhesion of parylene-C insulation layer.

3.4. Immunohistochemical analysis

After the analysis of electrical functions over 12 weeks, we performed the tissue analysis by staining astrocytes and microglia, which mainly consist of a glial scar. Furthermore, we investigated the distribution of nuclei to identify neuronal cells. Five electrode sites were examined for immunohistochemical analysis. Figure 8(a) shows an example of stained brain tissues. The diameter of the electrodes was identified to be about 25 μm from the brain slices.

Figure 8(b) shows the normalized fluorescence intensities according to the distance from the center of electrodes, as an indicator of the distribution of cell types. The spatial distribution of astrocytes increased up to 40 μm from the electrode center and then decreased. The distribution of microglia was the highest at 20 μm to 25 μm from the electrode center and was stabilized at distances greater than 100 μm. The existence of microglia and astrocytes surrounding the electrodes represents the glial scar formation that interferes the neuronal signal recording. The fluorescence intensity of DAPI represents the presence of nuclei, including astrocytes, microglia and neurons. Although we did not stain neurons exclusively, we could estimate the distribution of neurons indirectly through the fluorescence intensities of GFAP, Iba1, and DAPI. If neurons were uniformly distributed along the distance, the DAPI intensity would be similar to the combination of GFAP and Iba1 intensities. However, the DAPI intensity did not change according to the distance from the electrode center, indicating that a relatively low number of neurons would be present around the electrode, compared to a region distant from the electrode, due to the glial scar formation.

The immunohistochemical analysis of the FPMA was compared with other intracortical microelectrodes, which are the UEA, shank-type silicon-based microelectrodes, and microwires. In a previous study using the UEA, the normalized GFAP density was increased up to 80–100 μm [38]. In studies using shank-type microelectrodes and microwires,
Figure 8. (a) Confocal fluorescence image of a stained brain section, in which astrocytes (GFAP, red), microglia (IbaI, green), and nuclei (DAPI, blue) are visualized. The white circle in the center represents the location where an electrode had been present. (b) Normalized fluorescence intensities of GFAP, IbaI, and DAPI obtained from five electrodes are shown as a function of the distance from the electrode center.

the fluorescence intensity of GFAP was increased from the electrode up to 100 µm [57, 58] while the fluorescence intensity of GFAP in the present study was increased up to 40 µm. It was reduced to 80% of its maximum at a distance of about 145 µm in our study, while in [57, 58], at a distance of about 250 µm. From this comparison, it can be concluded that the formation of glial sheath layer around the FPMA was less than or at least comparable with the previous studies on other intracortical electrodes.

4. Conclusion

The electrical performance of the FPMAs was assessed for 12 weeks by implanting them in the somatosensory cortex of rats. Generally, the FPMAs exhibited increasing impedance and decreasing AP amplitude for the first 4 weeks after implantation, indicating the presence of acute biological responses. Such acute responses depended on the implanted environment of individual animals and the used surgical techniques significantly. After 4 weeks of implantation, we observed gradual declines in AP amplitude, accompanied by either slightly increasing impedance caused by chronic tissue responses such as glial sheath formation and neuronal degeneration, or decreasing impedance caused by the delamination of insulation layer. Despite the biological responses at the implant site or the delamination of insulation layer of the FPMAs, it was demonstrated that the electrodes of the FPMAs successfully recorded neuronal signals for 12 weeks with stable signal quality, which was verified by the SNR maintained consistently over time.

Data availability statement

The processed data required to reproduce these findings cannot be shared at this time due to technical limitations of a massive data capacity. The authors confirmed that the data supporting the findings of this study are available within the article and its supplementary materials.

The data that support the findings of this study are available upon reasonable request from the authors.

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