別紙４－１（課程博士（英文））

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Abstract (Doctor)

Title of Thesis	Nanoscale-tipped microwire array devices for in vitro and in vivo intracellular applications

Approx. 800 words

Nanoscale structures including nanowires and nanotubes are used in various fields such as sensors, solar cells, batteries, and biological applications. Especially, intracellular applications such as electrical measurement and deoxyribonucleic acid (DNA) introduction with minimally invasive are expected. In recent years, various intracellular devices have been reported and contributed greatly to neuroscience and medicine fields. Intracellular signal recording by penetrating a nano-scale electrode into the cell has an advantage of larger signal amplitude (~100 mV) compare to the extracellular signals (~100 μV). Moreover, intracellular recording obtains the signals below the threshold of the action potential such as the excitatory postsynaptic potential (EPSP) or inhibitory postsynaptic potential (IPSP) which cannot be observed by extracellular recordings. In addition, the DNA introduction method using a nanoscale device has an advantage of local area injection with minimizing damage to the cells. However, these nanodevices cannot be used for thick biological samples such as brain slices and brain tissues *in vivo*, due to the short wire length of less than 10 μm.

In this research, it is aimed to develop a nanoscale wire device with the wire length of more than 100 μm, which length enables intracellular electrical measurement and DNA introduction into cell for thick biological tissues including brain slice and brain *in vivo*. In order to realize the 100-μm long nanowire devices, I have proposed the nanoscale-tipped silicon microwire device by sharping the tip portion of the silicon microwire, which can be vertically assembled on a silicon substrate by gold-catalyzed selective vapor-liquid-solid (VLS) crystal growth of silicon.

As the first step, mechanical characteristics of the nanoscale-tipped silicon microwire with the length of more than 100 μm were quantitatively evaluated by finite element analysis. Compared to cylindrical shaped nanowire (tip diameter 500 nm, electrode length 150 μm), the proposed cone-like shape (tip diameter 500 nm, terminal diameter 10 μm, electrode length 150 μm) wire shows stiffness of 4.09 N/m, which is about 7,500 times larger than that of cylindrical one (5.2×10^{-4} N/m). The calculation results also indicated that the stiffness of the proposed wire is high enough to penetrate brain tissue. In addition, in order to realize simultaneous multi-point electrical intracellular recording, it was necessary to electrically insulate between the nanowire-electrodes. Therefore, it was proposed that the wires were grown on a silicon on insulator (SOI) substrate. By combining these preliminary studies, arrays of nanoscale-tipped microwire electrodes (NTE) array devices with wire-lengths of 140 μm to 400 μm were fabricated.

In order to explore the intracellular recording capability, electrical characteristics of the fabricated NTE was evaluated. The electrode impedance ranged from 895 MΩ to 305 kΩ at 1 Hz to 10 kHz (2.3 MΩ at 1 kHz for action potential recroding), which were similar to conventionally used glass pipette electrodes. The output/input signal amplitude ratio of the NTE during intracellular recording was 85% to 37% at 1 Hz to 10 kHz, while the noise levels showed 5 $mV/Hz^{-1/2}$ to 15 $nV/Hz^{-1/2}$ at 1 Hz to 10 kHz. From these results, intracellular potentials of ~ 100 mV can be obtained with the high signal-to-noise ratio using the fabricated NTE device.

In vitro intracellular recording from mouse's muscle (tibialis anterior muscles) was demonstrated using fabricated 200-μm-long NTE devices. During the NTE penetration into the cells, voltage changes ranged from 19 mV to 35 mV were confirmed before and after the penetration. This result indicated that the fabricated NTE penetrated the cells and measured the resting membrane potentials of cells. As a next step, *in vivo* extracellular recording was demonstrated using mouse's brain. An array of 200-μm-long NTEs recorded neuronal signals from the barrel field (somatosensory field). The recorded signals were local extracellular potentials, which were evoked by the mouse's whisker stimuli. *In vivo* intracellular recording was also demonstrated using mouse brain (barrel field). A NTE with the length of 400 μm measured voltage changes from 52.9 mV to 98.2 mV, indicating that the proposed NTE was inserted into the cell and measured the resting membrane potential.

As another application of the nanoscale-tipped microwire, *in vivo* multiple-cell DNA injection in the biological tissue was proposed. The fluorescent expression vectors of a marker protein (Venus) were injected into the barrel areas of mouse brain *in vivo* using a 200-μm-long NTW array. Cleared whole brain two days after the injection was observed using the two-photon microscopy, and fluorescence was confirmed at a depth of about 200 μm from the brain surface. This result indicates that DNA was injected into cells in the brain *in vivo*.

These studies demonstrated important intracellular applications including intracellular signal recording and cell DNA injection in the deep area of the brain tissue, which were limited by conventional methods. Especially, the multisite intracellular recording has been limited to cultured cells. I hope that this research will contribute to accelerating the measurement for brain slice and brain tissue.