Biocompatibility of a quad-shank neural probe

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ABSTRACT

Multichannel, flexible neural probes have been fabricated using standard CMOS techniques. The neural probe consists of four shanks with 16 recording sites each of approximately 290 μm². The recording sites are created using gold rectangular pyramidal electrodes sandwiched between two polyimide dielectric layers. Windows in the first polyimide layer expose the electrode sites and bonding pads. The bonding pads and interconnect wires at the topmost section of the probe are soldered to tungsten wire followed by encapsulation with epoxy to protect the interconnections from contact with phosphate buffered saline solution. The electrode test impedance values at 1 kHz are on average 135 kΩ. Multi-walled carbon nanotubes (MWCNTs) were deposited on electrode sites resulting in a reduction of impedance at 1 kHz to 6.89 kΩ on average. Moreover, the cell viability and proliferation of the PC12 cells on the surface of the probe was investigated by trypan blue exclusion assay to evaluate biocompatibility of the probe material. The PC12 cells attached and grew on the surfaces of the probe with no significant effect on the cells’ morphology and viability. The polyimide probe displayed a good cell viability and proliferation, making the polyimide attractive for potential candidate as probe materials in the fabrication of neural probes.

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1. Introduction

Neurotechnological tools have enabled the acquisition of data to help describe the complex circuitry of the brain, especially in the characterization of neurodegenerative diseases. Over time, neurotechnological tool design has gradually evolved from the conventional patch-clamp to microelectrode array technology to improve the accuracy/ resolution of both electrical and chemical recordings obtained from the electrode sites. Neuroscience has recently taken advantage of the standard silicon micromachining used in the fabrication of CMOS devices to fabricate micro- and nanoscale neural probes to monitor brain activity. This emerging field, known as neural microelectromechanical systems (neuroMEMS), holds great promise of less invasive, high fidelity recording of brain activity [1–3].

Biocompatibility, probe stiffness and tissue damage are major concerns in the development of new neural probes for acute and chronic implantation studies [4]. This can be attributed to the decisive factors such as the material composition, mechanical properties, sterilization processing and so forth of the neural probe that comes into intimate contact with the neural tissue and provokes a natural immune response. The elicited immune response can then cause the neural probe electrical/signal characteristics to deteriorate overtime. Thereby, new neural probe material must be able to withstand the biological conditions inside a living organism over a long period of time. Additionally, tissue damage ensues in the case of the implantation of rigid neural probe shank due to the brain’s micromotions. Future generations of neuroMEMS probes seek to address these problems by dramatically decreasing the footprint of each neural probe through microfabrication and MEMS technologies. There are two neuroMEMS probe arrays commonly used for recording the electrical activities in the brain; the Michigan probe and the Utah probe. Both probe arrays are silicon based, with probe thicknesses as little as 15 μm. Both arrays have distinct advantages and disadvantages. The Michigan probes have low mechanical strength and require a special guide tool for insertion, whereas the Utah probes are limited in the length of the probe. This limitation is due to the fact that the shaft height in the Utah probe is realized using subtractive fabrication process and is dependent on the thickness of the silicon wafer utilized. Despite the small footprint of neural probes, silicon-based probes are hypothesized to elicit some degree of tissue damage when implanted in the brain due to the stress-strain caused by the interaction between the probe and the brain’s micromotion. Therefore, probe fabrication research has shifted its focus to employing biocompatible polymers due to their biocompatibility and enhance mechanical compliance to reduce the chronic immune response.
and tissue damage by minimizing the strain–stress caused by an interplay between the probe and the brain's micromotion. Polymers such as polyethylene-c, polydimethylsiloxane (PDMS) and polyimide have long been used as biocompatible coating of silicon based probes, but more recently they have been studied as both insulation layers and interconnect cables that replace more rigid silicon and metal based components [5–7].

In addition to brain tissue damage, brain tissue has been found to develop a glial sheath over probes during chronic implantation. This glial sheath is made up of microglia and astrocytes serving to protect neurons from foreign bodies. The sheath ultimately results in encapsulating and shielding the neural probe’s recording sites from recording the neuronal activities [8]. Very thin neural probe designs have been found to prevent glial sheath formation on implanted neural probes [9]. Another approach to mitigating gliarial encapsulation has been to modify the surface of the neural probe to control the level of adherence of glial or neural cells [10].

In this study, we present a novel, multichannel neural probe fabricated using standard microfabrication techniques with photopatternable polyimide (HD-4104 and HD-8820 polyimide (HD Microsystems, USA)) serving as the structural support and dielectric material. This new design includes 16 recording electrode sites on each of the 4 shank, thereby providing up to 64 recording channels that measures less 41 μm in width. Electrochemical impedance spectroscopy (EIS) [11] is used here to characterize the impedance response of the electrode in the quad-shank neural probe over a frequency range of 0.1 Hz–10 kHz, with 1 kHz being the physiologically relevant frequency. Cell cultures have been shown to provide a picture of biocompatibility in vitro [12]. Here, in vitro biocompatibility is assessed from cell viability and cell proliferation studies performed using cultured PC12 cell line derived from a pheochromocytoma of the rat adrenal medulla. These PC12 cells exhibit neuron-like properties in the presence of neuronal growth factor and make good analogs to brain tissue [12].

2. Experimental section

2.1. Materials

Ethanol, Phosphate Buffered Saline (KCl 0.20 g/L, KH2PO4 0.20 g/L NaCl 8.00 g/L, Na2HPO4·7H2O 2.16 g/L), acetic acid, laminin, Ham’s FK12 Cell Culture Media, polydimethylsiloxane (Sylgard® 84 silicon elastomer kit), trypsin-EDTA, trypan blue, were purchased from Fisher Scientific. The hemacytometer was purchased from Hauser Scientific Company. Adherent PC-12 cells were purchased from ATCC.

2.2. Probe fabrication

The flexible multi-shank neural probe is designed for enhancing neuro-interfacing performance. The neural probe has multiple metallization and passivation layers in which the 1 μm electrical interconnects are sandwiched in between the two photopatternable polyimide, HD-4104 and HD-8820 polyimide (HD Microsystems, USA) as illustrated in the cross-sectional diagram of the major fabrication process in Fig. 1. The contact pads and electrical interconnects are formed on a single gold metallization layer. The presented neural probe architecture is made of three metallization layers. The first metallization layer forms the 16 5 × 50 μm recording electrodes followed by a second metallization layer that defines the 1 μm interconnects that establish electrical continuity between the recording electrodes and the contact pads. These two metallization layers are separated by a thin polyimide film. Polyimide as a structural, dielectric, and passivation layer offers structural flexibility and good stability for neuron-electrode interface. The final gold metallization layer carries the exposed routing leads and the contact pads. Gold was chosen as the metallization material because of its chemical inertness, biocompatibility and compatibility with the microfabrication process.

2.3. Impedance spectroscopy

Impedance spectroscopy measurements were taken using a potentiostat PGSTAT204, Metrohm Autolab. Tungsten wires were bonded to each contact pad with conductive silver glue and each wire was connected one at a time to the bonding pad. The quad-shank neural probe was submerged in phosphate buffered saline (PBS) solution at room temperature and pH 7.4. The experiment was performed in a three-electrode cell configuration with a Ag/AgCl electrode as the reference electrode and platinum electrode with a surface area of 9.87 cm² as the counter electrode. The probe quad-shank was left in solution for 45 min before each impedance recording was made. Measurements were made in triplicates. The electrochemical impedance magnitude, and phase measurements were taken with a frequency range between 0.1 Hz and 10 kHz using a 10 mV peak-to-peak waveforms.

2.4. Multiwalled carbon nanotubes (MWCNTs) deposition

The gold recording site electrodes surface area can be increased by roughening the gold surface with multi-walled carbon nanotubes (MWCNTs) [13]. To coat the gold recording electrodes with MWCNTs, MWCNTs and gold sulfite solution (TSG-250) were purchased from Cheap Tubes Inc. and Transene, respectively. MWCNTs were dispersed into the gold electrolyte bath solution at a concentration of 1 mg/ml. The electrolyte bath was then sonicated for two hours in a sonication bath in order to disaggregate and suspend the MWCNTs in solution. A platinum wire electrode was used as the anode. Each of the electrodes were then connected to a function generator (Agilent 33250A) with a monophasic voltage pulse of 1.2 V, 10 Hz, at 50% duty cycle for 1-min duration. During electrodeposition gold particles absorbed onto the MWCNTs due to electrohoresis of the gold ions and resulted in shorter MWCNT-gold composites deposition on the gold recording electrode acting as a negative terminal [14]. Impedance spectroscopy was performed before and after each deposition and scanning electron microscopy (SEM) microscopy was used to further verify the deposition of MWCNTs.

2.5. Biocompatibility testing with cell culture

In general, before neural probes are implemented in vivo, the biocompatibility of the neural probe must be first accessed. Here we have taken the initial steps to verify the biocompatibility of the fabricated polyimide neural probe prototype recording electrodes exposed to PC12 cell line in vitro. A single probe was fixed at distal ends in 6-well culture plates. Two control plates were prepared by culturing PC12 cells on extracellular matrix protein (laminin) at a concentration of 3.5 μg/mL. PC12 cells were incubated in Ham’s FK12 at 37 °C and 5% CO2. The cells were harvested using 0.25% trypsin in EDTA. Subsequently, the cells were centrifuged and re-suspended in Ham’s FK12. A final cell suspension of 3 mL containing 1 × 10⁶ cells/ml was seeded on the polyimide probe and laminin treated surfaces. The morphology of the cells was examined by optical light microscopy after 48-h. After day 2, the cells were trypsinized, re-suspended, and counted to determine viability and proliferation via trypan blue staining.
3. Results and discussion

3.1. Neural probe microfabrication

The four shank neural probe was fabricated using top-down microfabrication approach on a silicon substrate. The optical micrograph of the completely fabricated and released polyimide neural probe (showing one shank) with gold metalizations is shown in Fig. 2. A high product yield of 95% was achieved for the release of the neural probes. The neural probe thickness is approximately 12 \( \mu \)m, which enables the probe to be very flexible and conform to its environment, thereby minimizing the potential risk of tissue damage [15].

Fig. 3 represents the scanning electron microscopy (SEM) micrograph of one of the shank with a length of 3 mm, thickness of \(~12 \mu\)m, and width of 41 \( \mu \)m, which is designed to allow neural recordings from deeper structures in the brain. The quad-shank neural probe consists of four shanks each equipped with 16 recording channels to enable the realization of up to 64 recording channels per neural probe layout. The Young’s modulus of the fabricated polyimide neural probe is \(12.5 \text{ GPa} \) (obtained via compression testing).

There is still a large discrepancy of Young’s modulus between the flexible probe and the brain tissue. However, the gap is reduced with the use of polyimide, thereby increasing the probe flexibility when compared to silicon based probes \((\sim 150 \text{ GPa})\). The polyimide isolates the bonding pads from the aqueous environment in the following \textit{in vitro} experiment. Tungsten wires were attached using conductive glue to the bond pads and connected to the impedance analyzer.

3.2. Impedance results

The quality of the neural probe signal is greatly affected by the electrode’s impedance [16]. On average, the \textit{in vitro} impedance magnitude of the recording electrode sites per shanks was frequency independent and had the lowest value over 6–10 kHz. In addition, the impedance magnitude was found to gradually increase with declining frequency [10] and the phase of the recording electrodes was approximately \(-60°\). The impedance value of 135 k\( \Omega \) \((n = 8)\) at 1 kHz of the gold rectangular pyramidal electrodes is attributed to the pyramidal geometry of the recording site electrode. However, the impedance can be significantly improved via the electrochemical deposition of platinum black, carbon nanotubes, or nanoparticles on the relatively smooth electrode surface. In addition, reducing the electrode’s impedance will increase the signal-to-noise ratio, which would further improve the accuracy of the neurophysiological results. Previous study by Xiang et al. showed that gold electrodes could be made more suitable for electrochemical application through the deposition of MWCNTs [14].

The deposition of MWCNTs resulted in significant reductions in impedance on each electrode tested. Fig. 4 compares an untreated gold recording electrode to a MWCNTs coated gold recording electrode using optical inverted light microscopy. The electrode surface before electrodeposition was relatively smooth and reflective. There was a significant change in the electrode surface after electrodeposition, where the surface appeared dark due to the MWCNTs coatings on its surface, thereby increasing the surface roughness and conductivity of the gold recording electrode. The average impedance of the electrodes was reduced to 6.89 k\( \Omega \) \((n = 8)\) at the physiologically relevant frequency of 1 kHz after electrodeposition. The impedance value of \(\sim 7 \text{ k}\Omega\) is well below most values reported for electrochemical sensing of neurotransmitters [17]. The relatively small impedance values obtained after the electrodeposition of MWCNTs is attributed to the large surface area of the pyramidal gold recording electrode design \((\sim 290 \mu\text{m}^2)\) to facilitate the gold nanoparticle absorption onto the MWCNTs, which was subsequently electrodeposited on the gold recording electrodes.
Fig. 2. Optical micrograph of fabricated neural probe: after device released from silicon substrate. Insert: Neural probe tip illustrating two gold recording electrodes and tip tapering to 3 μm in diameter.

Fig. 3. (A) Fabricated neural probe Array: Flexible neural probes were released in buffered oxide etchant, SEM image of the contact pads, routing leads, interconnects and recording and stimulation electrodes view of the neural probe with up to 64 recording and stimulation electrodes. (B) CAD of neural probe shank showing probe shape, contact pads and interconnects.

Fig. 4. Gold recording electrode before (left) and after (right) MWCNTs deposition. Insert: SEM micrograph of the electrodeposited MWCNTs.
3.3. Biocompatibility assay

Established cell lines are used regularly to validate implantable materials for cytotoxicity and cell proliferation, and thereby are used as an indication of possible scarring and histocompatibility [8,10]. After the 48-h culture period, the PC12 cells adhered, spread, and proliferated. The representative cell morphology as depicted on the various surfaces depicted in Fig. 5 ranged from spindle to spherical shaped. In most cases, the cells colonized the substrate surfaces and exhibited a spherical morphology, with the exception of the neural probe surface, which did not favor the colonization of cells on the polyimide surface.

Cells grown on the polyimide neural probe proliferate by 40% and the laminin treated surface exhibited an increase of 184%. However, the viability of cells in the presence of the neural probe did not significantly decrease and was measured at 98.58% viability. Fig. 6 compares the cell density after 48 h of each trial, while Fig. 7 compares the proliferation measured as a percent difference from the starting density. Fig. 8 compares percent viability. As illustrated in Figs. 7 and 8, cells grown on PDMS were not able to adhere to the substrate. As a result, cell proliferation was significantly decreased along with viability. Proliferation decreased by 44% while viability decreased from 98% to 91%. The laminin treated surface did not show a significant difference in proliferation or viability when compared to the bare cell culture well control. The cells proliferated at 184% and had a modest 1% increase in viability.

These observed results revealed that the polyimide probe was suitable for the proliferation of the PC12 cells. Importantly, no negative effect on the cell growth was observed for the polyimide. Importantly, the gold metalization layer sandwiched between the two polyimide layers remained intact under physiologic conditions, thereby maintaining the effective surface area of the MWCNTs-gold recording electrodes.

4. Conclusion

Our results demonstrate that the polyimide neural probe as designed and fabricated promotes adequate cell growth and proliferation. The neural probe exhibits a relatively improved overall recording electrode impedance without roughening of the electrode surface. Further treatment of the electrode surfaces with gold nanoparticles absorbed onto the MWCNTs improve the effective...
surface area. Thereby, the as fabricated neural probe hold promise for measurement of chemical signals in the brain. The cell viability assay reveals a slow proliferation of PC12 cells on the polyimide neural probe surface with low cell death, which is highly desirable for in vivo implementation.

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References


Fig. 7. PC-12 Adh cell proliferation percentage after 48 h of grown on laminin, polyimide probes and PDMS treated surfaces.

Fig. 8. PC-12 Adh cell proliferation percentage after 48 h of grown laminin, polyimide probes and PDMS treated surfaces.

Joel Tyson is currently pursuing the Bachelor's degree in chemical and biochemical engineering at the University of Maryland Baltimore County. He is involved in the development of the flexible quad-shank neural probe for neurotransmitter and local field potential monitoring using carbon nanotube decorated electrodes. His current research interests include domains of bioengineering, electrochemical characterization, neural prostheses and BioMEMS.

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