Printable and Transparent Micro-electrocorticography (μECoG) for Optogenetic Applications

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***Abstract*—Micro-electrocorticography (μECoG) displays advantages over traditional invasive methods. The μECoG electrode can record neural activity with high spatial-temporal resolution and it can reduce implantation side effects (e.g. vascular and local-neuronal damage, tissue encapsulation, infection). In this study, we propose a printable transparent μECoG electrode for optogenetic applications by using ultrasonic microfluid printing technique. The device is based on poly(3,4-ethylenedioxythiophene): poly(styrenesulfonate) (PEDOT: PSS) as a conductive polymer, polydimethylsiloxane (PDMS) as an insulating polymer and poly(chloro-para-xylylene) (Parylene-C) as the device substrate. We focus on ultrasonic microfluid printing due to its low production cost, excellent material handling capability, and its customizable film thickness (down to 5-20 microns). The ultrasonic fluid-printed μECoG displays high spatial resolution and records simulated signal (0-200 Hz sine wave) effectively with low electrode impedance (50-200 kOhms@1kHz). The μECoG also shows good biocompatibility suitable for customizable chronic implants. This new neural interfacing device could be combined with optogenetics and Brain-Computer Interface (BCI) applications for a possible future use in neurological disease diagnosis and rehabilitations.**

I. Introduction

In 2006, World Health Organization (WHO) reported that neurological disorders affect over one billion people worldwide [1]. In some cases, traditional treatments such as therapeutic drug and neurosurgery cannot be used effectively. Brain-Computer Interface (BCI) has been rapidly developed for neurophysiological solutions especially in bidirectional feedback field. Optogenetics is a new technology that promotes BCI evolution. This approach is a neuromodulation that uses specific wavelength of light to control light-sensitive membrane protein (opsin). This method achieves selectively neural control at high spatial-temporal resolution on millisecond timescales at cellular level [2], [3]. The unique characteristics of optogenetics enable two-way neural interfacing where both recording and stimulating neurons in cellular level can be achieved.

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The micro-electrocorticography (μECoG) can be used for the neurophysiology with optogenetics. The μECoG electrode, which is placed on the brain surface, can record high gamma band activity and displays advantages over other invasive methods such as single unit microelectrodes and traditional ECoG electrodes [4]. The μECoG does not penetrate the brain tissue, so this method can reduce vascular and local-neuronal damage, CNS infection and tissue encapsulation. Moreover, the μECoG electrode has higher spatial-temporal resolution and accuracy than electroencephalogram (EEG). For these reasons, it can improve neural interfacing for diagnosis and treatment. The most common fabrication technique of high-resolution μECoG is microfabrication that can create micro-scale features with lift-off process, plasma etching and photolithography [5-7]. These methods usually require the cleanroom environment that has some disadvantages, such as expensive process, complex systems and time consuming.

In this project, we propose the printable and transparent μECoG electrodes for optogenetic applications. The ultrasonic fluid printing is used to deposit electrical connecting and insulating layer of the device. The ultrasonic fluid printing has many advantages over the microfabrication technique, including high feasibility, low cost, material efficiency with versatile fluid handlings, simple manufacturing process and low time consuming. This printing technology can generate droplets and continuous patterns in microscale with great precision [8]. PDMS, the highly transparent, inert and biocompatible polymer, was diluted by *tert*-Butanol [9] and printed as insulating ink. PEDOT:PSS, the most stable commercially available conducting polymer, was used as conductive ink. It displays good film printing property, good biocompatibility and thermal stability. However, PEDOT:PSS film is hydrophilic. Nafion, a transparent and biocompatible ion-exchange polymer, is an additive which can improve water resistive and surface immobilization of PEDOT:PSS film [10]. These polymers were deposited on transparent and biocompatible Parylene-C substrate [11] to form the complete 3-layer μECoG electrodes. The device's electrical properties, mechanical characteristics, and cytotoxicity were evaluated to determine its future as implantable neural interface.

II. Materials and Methods

A. Characterization of PEDOT:PSS (0.5%Nafion)

The 0.8% PEDOT:PSS ink (Sigma-Aldrich, Product No. 739316) was sonicated by ultrasonic bath for 20 minutes and filtered with 0.2 μm nylon filter to avoid nozzle clogging. The 5% Nafion (Sigma-Aldrich, CAS No. 31175-20-9) was

diluted with deionized distilled water to obtain 0.5% Nafion. The 0.5% Nafion solution was added into the PEDOT:PSS ink at the volume ratio 7:3, 1:1 and 3:7. All solutions were stirred at room temperature for 24 hours, then dropped on Parylene-C coated PET and annealed at 60°C for 8 hours. Four points probe unit was used to measure sheet resistivity of PEDOT:PSS (0.5%Nafion) film by passing current through two outer probes and measuring voltage between two inner probes.

B. The μECoG Design

The SonoDraw software (SonoPlot, Inc.), vector-based CAD tool, was used to design electrode patterns before printing by GIX Microplotter II instrument. The proposed electrode consists of conductive circuit (to record electrical signal from target areas) and insulating layer (to protect the conductive circuit from the short circuit, noises and environment). The μECoG has three layers that are Parylene-C on the bottom, PEDOT:PSS (0.5%Nafion) in the middle and PDMS on top.

The conductive circuit starts from electrode sites that are placed on the brain surface. With the electrode traces, signal can be sent to the amplifier unit via contact pads and PCB. In this project, there are two different types of electrode. One with all-printed PEDOT:PSS (0.5%Nafion) (volume ratio 1:1). To increase its mechanical stability the silver nanoparticle ink was printed over the PEDOT:PSS (0.5% Nafion) in the contact pad areas. The other type has a platinum adapter up to electrode neck (pink area in Fig. 1). To achieve the electrode site with 200 μm in diameter, the SonoDraw design for each electrode site consists of three circles with diameter 25, 65 and 100 μm. The distance between each site from the center is 750 μm. These microelectrodes have 17 traces and sites for 16 recording channels and one reference. For the insulating layer, it was designed to cover the conductive pattern except the electrode sites and PCB contact pads.

C Polymer Ink Preparation

Three types of ink were prepared. The preparation process of PEDOT:PSS (Nafion) (volume ratio 1:1) was described in characterization of PEDOT:PSS (0.5%Nafion) in section A. For diluted PDMS ink, the *tert*-Butanol (Sigma-Aldrich, CAS No. 75-65-0) was warmed at 45°C and mixed with PDMS (Dow corning, CAS No.68988-89-6) and SYLGARD 184 curing agent (Dow corning, CAS No. 63394-02-5) at the weight ratio 50:10:1. The diluted PDMS was mixed by vortexer for 15 minutes. For silver nanoparticle ink, the TPS HS silver nanoparticle ink (Bayer) was stirred by the vortexer for 15 minutes.

D. Micropipette Tip Assembly

To get the customized micropipette tips (25μm), the Borosilicate capillary tubes (Fisher brand) were pulled by Flaming Brown Micropipette Puller MODEL-P87 (Sutter Instrument). Then these tips were attached with the piezoelectric of dispensing cartridges. We customized parameters for pulling the tips and we obtained the tips with the average diameter of 23.392 ± 1.315μm.

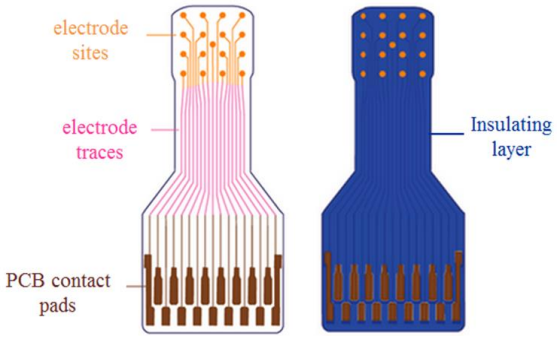

Figure 1. The design of conductive circuit (left) and insulating layer (right)

E. Parylene-C Substrate Preparation

The clean 3-inch silicon wafer was coated with Parylene-C (SCS, CAS No. 28804-46-8) by Specialty Coating Systems PDS2010 coater. To get 15 μm thicknesses, it is required to use approximately 25 grams the Parylene-C dimer. The Parylene-C coated silicon wafer has to be improved the hydrophilicity before printing by the oxygen plasma surface system (Labor-und Kleinserienanlage FEMTO (Niederdruck-Plasma). With oxygen plasma treatment, the continuous pattern can be printed with higher hydrophilic surface. The oxygen flow rate was set at 35 sccm, pressure of 2 bars, plasma power of 50 Watts and treat for 30 seconds in the oxygen plasma process.

F. The μECoG Fabrication

SonoGuide was used to control the GIX Microplotter II which can dispense picoliter of inks precisely. The micropipette tips should be cleaned by pumping and spraying of Isopropyl alcohol (IPA) before filling polymer ink. Next steps are dispensing calibration origin setting, surface finding and surface cant calibration respectively. Different ink can be dispensed with different voltages and time following Table I.

For all-printed microelectrode, the PEDOT:PSS (0.5% Nafion) was printed as conductive circuit and anneal at 60°C for 8 hours. The TPS HS nanosilver ink was printed on PCB contact pads and anneal at 100°C for an hour. Then, the diluted PDMS was printed to cover the conductive circuit except electrode sites and PCB contact pads and annealed at 60°C for 2 hours to crosslink PDMS film.

The microelectrode with adapter, the PEDOT:PSS (0.5% Nafion) was printed on Parylene C coated silicon wafer as conductive circuit around the top of microelectrode which has electrode sites and annealed at 60°C for 8 hours. Then, the diluted PDMS was printed as insulating layer cover conductive circuit except electrode sites and PCB contact pads and annealed at 60°C for 2 hours to crosslink PDMS.

TABLE 1. PRINTING PARAMETER OPTIMIZATION

Polymeric Ink	Average Voltage (V)	Average Time (s)
PEDOT:PSS(0.5%Nafion)	5.3 ± 0.4	2 ± 0.5
Silver Nanoparticle	2.6 ± 0.2	1 ± 0.5
Diluted PDMS	6 ± 0	2 ± 0.5

The microelectrodes were cut by using GRAPHTEC Craft ROBO Pro, soaked in DI water and dried in air. The devices were attached with Polyimide sheets in area of PCB contact pads to match with PCB connector gap by the following processes. The adhesive layer of Polyimide sheets was attached on the backside of the contact pads. They heated between two aluminum foil sheets and compressed by the load cell with pressure 280 PSI and heated at 180°C for 1.5 hours. After the heater was cool down, the devices were removed from the foil.

G. Electrode Impedance

To evaluate the recording quality of our electrode, we measured the electrode impedance. The electrode connected PCB and the ground wire were immersed into the saline. Impedance spectrum information was measure by a potentiostat (Autolab PGSTAT12).

H. Signal Stimulation

To make sure that our electrode can measure cortical signal frequencies accurately, we generated sine wave from 0-200 Hz via a wire and assessed recording signal from our electrodes. The electrode with the reference, ground wire and the signal-generated wire were immersed into saline. The frequencies and amplitudes were recorded with TDT Bioprocessing system.

I. Cell Culture

Murine fibroblast cells (L929) were provided by the STEn LAB, Mahidol University (Thailand). This cell line was cultured in Roswell Park Memorial Institute (RPMI) 1640 with 55 (v/v) FBS in a humidified atmosphere containing 5% CO_2 at 37°C.

J. Cytotoxicity Studies of µECoG

The *in-vitro* cytotoxicity of µECoG was investigated with two methods. First, direct contact testing of µECoG was evaluated to confirm cell adhesion and proliferation of the electrode. The tip of the µECoG electrode was sterilized by 70% ethanol for 15 minutes. The sample was then washed twice with 1 ml of phosphate buffer saline (PBS). L929 cells were placed on the sample in 96-wells plate for 24, 48 and 72 hours. Second, indirect contact test was prepared by incubating L929 with an extracted medium obtained from the incubated the electrode with RPMI 1640 for 3, 7 and 14 days at 37°C. The cell viability was observed using the MTT standard procedure at wavelength 550 nm (TECAN M200pro).

III. Results and Discussion

A. PEDOT:PSS (0.5%Nafion) Sheet Resistivity

Average sheet resistivity of PEDOT:PSS, PEDOT:PSS (0.5%Nafion) (3:7), PEDOT:PSS (0.5%Nafion) (1:1) and PEDOT:PSS (0.5%Nafion) (7:3) thin film are 73.502 Ω/□ ± 1.564, 76.167 Ω/□ ± 4.914, 79.866 Ω/□ ± 6.435 and 85.22 Ω/□ ±15.361, respectively. From the results, PEDOT:PSS (%0.5Nafion) (3:7) and PEDOT:PSS (0.5%Nafion) (1:1) thin film have sheet resistivity similar to PEDOT:PSS thin film. Although, PEDOT:PSS (0.5%Nafion)(3:7) thin film showed lower sheet resistivity, but it has more Nafion which can increase the viscosity. Therefore, PEDOT:PSS (0.5%Nafion) (1:1) is more suitable for conductive ink with high electrical conductivity and low viscosity.

B. Printing Result

The final printing results show that the ultrasonic fluid printing can print continuous traces with average diameter of 45.84 ± 12.76 µm, electrode sites with average width of 210.85 ± 21.43 µm, Fig. 2. All channels were separated to each other completely. Therefore, this technique can be applied for microelectrode fabrication with high resolution that is suitable for signal recording.

C. Impedance Spectra Result

The impedance of 10 channels from all type of electrodes at frequencies of 20-30,000Hz was measured. Average impedance from the microelectrode with adapter is acceptable at ~ 1 kHz at 72.917 ± 28.07 kΩ. Average impedance at ~ 1 kHz from the all-printed microelectrode is 125.35 ± 47.90 kΩ which is slightly higher than the other type, Fig. 3. However, the impedance spectrums from both types are acceptable with respect to conventional standard.

D. Signal Stimulation Results

The microelectrode with adapter was used to record 0-200 Hz sine wave. The stimulation results show that we can get accurate recording signal from the microelectrode with adapter. Therefore the microelectrode with adapter can be used to record cortical signal in the common range for µECoG electrode (0-200Hz) as shown in Fig. 4.

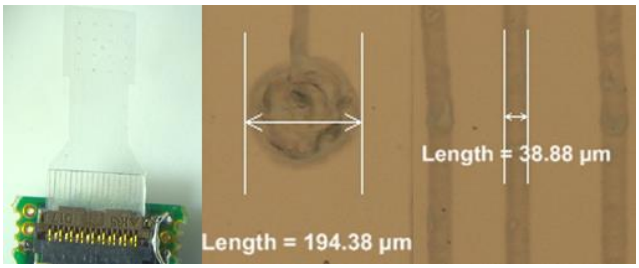

Figure 2. The all-printed transparent microelectrode connected to PCB connector (left), and electrode site and traces (right).

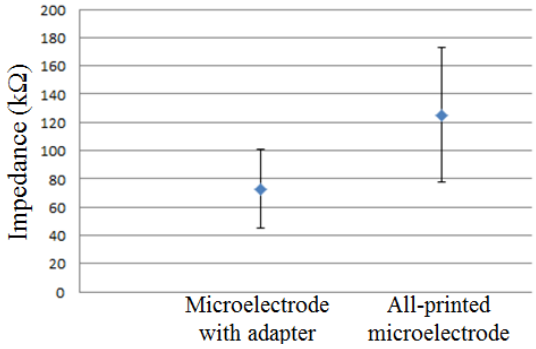

Figure 3. The impedance of printed electrodes (n=10) at ~ 1 kHz

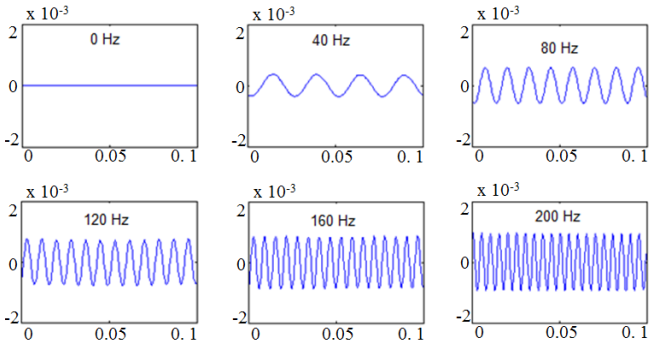

Figure 4. Recording signal from microelectrode with adapter (X axis is time (second), Y axis is voltage (volt)) after stimulation by 0-200 Hz sine wave.

E. Cytotoxicity Test

The proliferation of L929 cells in the extracted medium was observed at day 3 to evaluate the cytotoxicity. The viability of L929 with extraction medium obtained from cells incubated for 3, 7 and 14 day were 106.94 ± 2.50, 115.71 ± 10.82, and 119.97 ± 2.22%, respectively, Fig. 5. For direct contact testing, the cells were seeded on microelectrode and culture for 3 days. The cell morphology was the same as the control, Fig. 6. These results suggested that our microelectrode is biocompatible and unlikely to cause any biological response in long-term implantation.

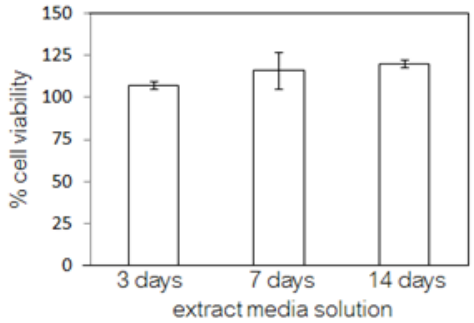

Figure 5. The cell viability after incubated with extraction medium from microelectrode after 3 days of cell culture.

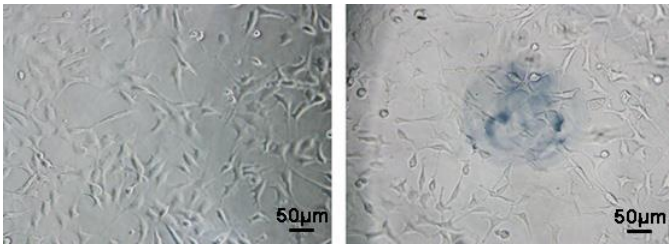

Figure 6. Morphology of L929 cells attached on (A) cell culture plate (control), (B) microelectrode, imaged by inverted microscope using a 20X objective lens.

IV. CONCLUSION

We can achieve suitable printing technique to fabricate high-resolution µECoG outside the cleanroom. The ultrasonic fluid printing can successfully fabricated transparent and printable µECOG electrode for optogenetic applications. This technique provides customizable µECOG with good biocompatibility and signal recording ability. The device can be used to record signal at different frequencies (0-200 Hz), including gamma band activity. It also showed low electrode impedance. At 1 kHz, the device has electrode impedance around 50-200 kΩ, acceptable for brain recording electrode. The cytotoxicity test suggests biocompatibility of our electrode before further *in-vivo* study. This study exhibits new way for microelectrode fabrication. In the future, the µECoG will be integrated with the micro light emitting diode (µLED) and implanted in optogenetic mice for long-term *in-vivo* study.

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REFERENCES

[1] World Health Organization (2006) NEUROLOGICAL DISORDERS public health challenges, Available at:http://www.who.int/mental_health/neurology/neurological_disorders_report_web.pdf (Accessed: 11th March 2014).

[2] Rein ML, Deussing JM. The optogenetic (r) evolution. Molecular genetics and genomics: MGG. 2012;287(2):95-109.

[3] Fenno L, Yizhar O, Deisseroth K. The development and application of optogenetics. Annual review of neuroscience. 2011;34:389-412.

[4] Moran D. Evolution of brain-computer interface: action potentials, local field potentials and electrocorticograms. Current opinion in neurobiology. 2010;20(6):741-5.

[5] Richner TJ, Thongpang S, Brodnick SK, Schendel AA, Falk RW, Krugner-Higby LA, et al. Optogenetic micro-electrocorticography for modulating and localizing cerebral cortex activity. Journal of neural engineering. 2014 Jan 20;11(1):016010.

[6] Thongpang S, Richner TJ, Brodnick SK, Schendel A, Kim J, Wilson J, et al. A micro-electrocorticography platform and deployment strategies for chronic BCI applications. Clin EEG Neurosci Of J EEG Clin Neurosci Soc ENCS. 2011;42(4):259.

[7] Schendel AA, Thongpang S, Brodnick SK, Richner TJ, Lindevig BDB, Krugner-Higby L, et al. A cranial window imaging method for monitoring vascular growth around chronically implanted micro-ECoG devices. J Neurosci Methods. 2013 Aug 15;218(1):121–30

[8] Bradley J. Larson, Susan D. Gillmor, and Max G. Lagally. Controlled deposition of picoliter amounts of fluid using an ultrasonically driven micropipette. Review of Scientific Instruments. 2004;75(4), pp. 832-836.

[9] John H. Koschwanez, Robert H. Carlson, Deirdre R. Meldrum. Thin PDMS Films Using Long Spin Times or Tert-Butyl Alcohol as a Solvent. PLoS ONE. 2009;4(2).

[10] Wen Y, Xu J, Li D, Liu M, Kong F, He H. A novel electrochemical biosensing platform based on poly(3,4-ethylenedioxythiophene) :poly(styrenesulfonate) composites. Synthetic Metals. 2012;162(13-14):1308-14.

[11] Rodger DC, Fong AJ, Li W, Ameri H, Ahuja AK, Gutierrez C, et al. Flexible parylene-based multielectrode array technology for high-density neural stimulation and recording. Sensors and Actuators B: Chemical. 2008;132(2):449-60.