Evaluation of a MEMS-based dual metal-layer thin-film microelectrode array for suprachoroidal electrical stimulation

Xiaohong Sui, Jingjing Sun, Liming Li, Chuanqing Zhou, Xuejiao Luo, Niansheng Xia, Yan Yan, Yao Chen, Qiushi Ren, Xinyu Chai

Abstract—A double metal-layer thin-film platinum microelectrode array was fabricated for implantation between sclera and choroid based on MEMS processing techniques and photosensitive polyimide material. The array was composed of 60 stimulating sites (6 × 10) and four selectable returning electrodes. The diameter of each stimulating electrode was 350 μm with a center-to-center spacing of 750 μm. The transient voltage responses of the electrode to current pulse stimulation indicated a charge-injection capacity greater than 52.1 μC/cm². Acute in vivo animal experiments showed that the implicit time of electrically evoked potentials (EEPs) was 17.09 ± 1.45 ms at a threshold current of 25.55 ± 5.43 μA for a full-row of simultaneously stimulated electrodes (i.e. current applied simultaneously to each of the 10 electrodes). Individual electrode stimulation threshold was 48.57 ± 6.90 μA. The corresponding threshold charge densities were 13.28 ± 2.82 μC/cm² and 25.24 ± 3.59 μC/cm², respectively. The spatial spread of the maximally recorded P1 response in the EEPs indicated a correspondence between the retinal stimulation site and the focal response location in the cortex. This method of array fabrication is suitable for acute suprachoroidal stimulation, and has a potential use for the fabrication of a visual prosthesis.

Index Terms—microelectrode, suprachoroidal implantation, electrically evoked potentials, visual prosthesis, polyimide film.

I. INTRODUCTION

More than 70% of the information humans use to recognize their surroundings comes from vision, thus blindness is a serious disability that leads to enormous challenges in daily life and limits the jobs available for visually impaired people. There are approximately 140 million people with some form of visual-impairment worldwide, and 45 million of these people are legally blind; a group that increases by 7 million each year. The main causes for a loss of vision are outer retinal degenerative diseases such as retinitis pigmentosa (RP) and age-related macular degeneration (AMD), which cannot be clinically treated by drug or surgical therapies.

A visual prosthesis [1] can activate neural cells by electrical stimulation from an implanted neural stimulator. Visual prostheses are classified according to the implanted location of the stimulating electrode array, for example, sub- and epiretinal, optic nerve, or cortical. A microelectrode stimulating array directly interfaces with the neural tissue, and is a key component of a visual prosthesis. The development of retinal prostheses has attracted more and more interest worldwide due to the rapid development of thin-film flexible microelectrode arrays based on micro-electro-mechanical system (MEMS) processing techniques. These arrays can be implanted into epiretinal [2, 3], subretinal [4] and suprachoroidal [5-7] anatomical locations to activate directly or indirectly retinal neural cells. A restricted list of flexible substrate materials for MEMS-based electrode arrays of retinal prostheses includes polyimide (PI) [1, 2], parylene-C [8], liquid crystal polymer (LCP) [6] and silicone rubber [7].

The various kinds of flexible microelectrode arrays for visual restoration must be fabricated according to the implant location and be evaluated prior to clinical trials. This paper reports on the microfabrication, in vitro electrochemical characteristics, and acute electrophysiological evaluation of a novel dual metal-layer microelectrode array based on photosensitive polyimide material. This dual metal-layer arrangement is beneficial in terms of saving space for the interconnecting lines of the 60 stimulating and 4 returning electrodes. In addition, the width was halved by arranging the lines as a 2 × 32 array. The array was implanted between the suprachoroid and sclera region for the purpose of indirect retina stimulation. Because the stimulating and returning electrodes are on the same substrate of the thin-film array, an additional returning electrode in the vitreous body was eliminated. The above factors reduced the amount of surgical damage to ocular tissues when using the array. Acute in vivo electrophysiologically animal experiments, in which electrically evoked potentials (EEPs) were recorded from the primary visual cortex (V1), were used to test the feasibility and
correspondence between the stimulating electrode and the focal location in the cortex after suprachoroidal implantation of this novel MEMS electrode array.

II. METHODOLOGY

A. Polyimide-based Implantable Microelectrode Array

1) Architectural design

The fabricated microelectrode array (Fig. 1a) was composed of 60 (6 × 10) circular stimulating sites and 4 Φ-800 µm independent returning electrodes. The stimulating-site region was 4.1 mm wide and 7.1 mm long. A single stimulating site was Φ-350 µm with a center-to-center spacing of 750 µm. All corners of the array were curved to reduce damage during implantation. The stimulating electrodes were connected to 4 lines of square 500 by 500 µm bonding pads. Two Φ-800 µm circular holes were developed for suturing the electrode array to the sclera for in vivo stability. The stimulating sites and returning electrodes were numbered in Fig. 1b.

2) Microfabrication

![Fig. 1. Prototype of the dual-metal-layer thin-film microelectrode array with 60-stimulating sites and four selectable returning electrodes. (a) The whole array structure. (b) Schematic illustration of the sixty stimulating sites and four returning electrodes. (c) The electrode array is viewed from the corneal side of the rabbit eye after implantation in the suprachoroidal space.](image)

Photosensitive polyimide (Durimide 7510, Arch Chemicals, Norwalk, CT, USA) was utilized to fabricate an implantable flexible thin-film electrode array. The manufacturing procedures (Fig. 2) began with a single-sided polished silicon wafer (46 Institute of Electronic Science and Technology, Tianjin, China) and were described as follows: (a) The silicon wafer was soaked in 5% hydrofluoric acid solution for 5 min to remove the natural silicon dioxide layer, and then was cleaned by standard RCA criteria and dried at 200 ºC. A 1 µm thick aluminum layer was thermally evaporated to coat the silicon substrate and act as a sacrificial layer in the later release step. (b) A lower polyimide layer was spin-coated onto the silicon wafer and patterned to form the outline of the electrode array, and then the 10 µm thick polyimide film was obtained after heated to 350 ºC in a nitrogen atmosphere. (c) A lower Ti/Pt (100 Å/1500 Å) layer was sputtered onto the array, patterned by lift-off technology, and adopted as stimulating sites, interconnecting lines, and bonding pads material. (d) A middle polyimide layer was then spin coated on top of the lower Ti/Pt layer and patterned to expose simultaneously the outline as in step (b) and half the number of stimulating sites and bonding pads. This layer was 5 µm thick after heated as in step (b). (e) An upper Ti/Pt layer was developed according to the procedure in step (c) for the same purpose. The double metal layer arrangement can save space for the interconnecting lines (64 parallel lines potentially occupy a large area, and by arranging the lines as 2×32, this area was halved). Moreover, the dual metal-layer arrangement can appropriately match flexibility with the mechanical stiffness of the thin-film electrode array for inserting into the suprachoroidal space. (f) An upper polyimide layer was spin coated on top of the upper Ti/Pt layer using the same process as step (d). (g) The upper polyimide layer was then patterned with an outline as in step (b) and the whole number of stimulating sites and bonding pads exposed. This layer was 5 µm thick after heated as in step (b). (b) The wafer was dipped in hydrochloric acid diluents for 3 h, to release the microelectrode arrays from the silicon substrate. Finally, the flexible thin-film microelectrode array was packaged to connect with an external current stimulator. The packaging procedures are described as follows: 1) plumbum-free solder was used to bond the bonding pads to enameled microwires; 2) epoxy resin AB glue (PC-CLEAR liquid epoxy, Protective Coating Co, Allentown, PA USA) was used for encapsulation to physically protect the bonding pads; 3) medical-grade silicone adhesive (SDG-A (M), Shanghai Institute of Rubber Products, Shanghai, China) was used to sandwich the bonding-pad area of the microelectrode array.

B. In vitro Electrochemical Characteristic Testing

1) Impedance

The electrochemical impedance was tested in vitro in phosphate buffered saline (PBS) solution using a precision LCR meter (Agilent E4980A, Agilent Technologies, Santa Clara, CA, USA) and three-electrode testing equipment.
including a working or stimulating electrode, a large flat platinum counter electrode with a surface area of 8 \times 10^{-2} \text{ cm}^2, and an Ag/AgCl reference electrode. An AC 50 mVpp voltage was applied with a frequency range of 20 Hz to 100 kHz.

2) In vitro current pulse stimulation

When using current stimulation, the transient voltage response of the stimulating electrode is a direct indicator of charge injection capacity. The transient potential responses were produced when current pulses were applied in a three-electrode configuration comprising an Ag/AgCl reference electrode, a large-area Pt mesh counter electrode, and the working or thin-film stimulating microelectrode (Fig. 3a). The electrolyte in the chamber was PBS solution. Stimulating current pulses were generated by an isolated and programmable current source stimulator (MS16, Tucker-Davis Technologies, Alachua, FL, USA). The stimulus waveform was a symmetrically charge-balanced cathodic-first biphasic pulse with a fixed pulse duration of 0.5 ms, frequency of 1 Hz, and an interphase delay of 0.17 ms. Stimulus current pulses of 10 - 100 μA were applied without bias potential. The linear voltage excursion of the electrode increased with an increase in the applied current pulse amplitude due to the capacitive double layer charging at the electrode-electrolyte interface [9]. The voltage drop induced by the PBS solution was subtracted to obtain the electrode voltage excursions. The charge injection capacity was calculated by determining when the most negative interphase potential exceeded a safe potential limit as defined by the water window [10].

C. Acute in vivo Electrophysiological Experiments

Five healthy adult albino rabbits (n=5, Fengxian, Shanghai, China), weighting 2.0 - 2.5 kg, were used in the experiments. All the experimental procedures were in accordance with the ARVO Statement on the Use of Animals in Ophthalmic and Vision research and were approved by the local ethics committee. The methods have been described in detail by Sun et al [11].

1) Surgical procedures

(a) Implantation of flexible microelectrode array

Two drops of a mixture of tropicamide (Tropicamide-DCPC, Double Cranes Pharmaceutical Co., Ltd., Beijing, China) and neosynephrine (Adrenaline Hydrochloride Injection, Harvest Pharmaceutical Co., Ltd., Shanghai, China) were used to dilate the pupil. The inferior conjunctiva was incised around the limbus, the outside canthal skin cut, and the eye maintained open by an eye speculum. The sclera was exposed by cutting the inferior rectus and oblique muscles and a 5 mm incision was made 10 mm posterior to the limbus to expose the choroid. The stimulating electrode array was implanted into the suprachoroidal space between the sclera and choroid with the aid of microforceps and positioned at the posterior pole of the eye, just below the optic nerve head; this position corresponded to the center of the visual streak. The implanted electrode array was photographed (Fig. 1c) by means of a contact lens on the cornea. After positioning the array, it was held in place by a non-absorbable 5/0 nylon suture connected to the sclera and rings on the device. Conventional fundus examination was used to determine whether any choroidal detachment or bleeding had occurred because of the surgery.

(b) Recording of EEPs

A recording electrode array made up of 5 × 6 silver-ball electrodes 0.3 - 0.4 mm in diameter with an inter-electrode center distance of 2 mm was placed on the exposed primary visual cortex (area 17, V1) contralateral to the stimulated eye in order to detect the EEPs responses (for details see also [11]). The impedance of the silver-ball electrodes ranged from 500 - 800 Ω measured by a LCR meter (E4980A,) using a 100 μA, 1 kHz AC current. A stainless-steel needle reference electrode was inserted into the forehead scalp ipsilateral to the stimulated eye, and a ground electrode placed subcutaneously in the ear tip.

The responses of V1 to suprachoroidal electrical stimulation were recorded on a 30-channel recording electrode array by a TDT system (System3, Tucker-Davis Technologies, Alachua, FL, USA). The threshold and effect of stimulating current strength versus EEPs amplitude at each channel were measured to determine stimulation characteristics and the spatial spread of the responses across V1.

2) Stimulating paradigm

The electrical stimulation from the programmable current source stimulator (MS16) was in a monopolar configuration with one Φ - 800 μm electrode as the returning electrode (Fig. 1b). The retina was stimulated using two configurations: 1) single electrode stimulation; 2) simultaneous full row electrode stimulation (i.e. current applied simultaneously to each of the 10 electrodes).

3) Data processing and analysis

Fifty consecutive cortical EEPs were recorded by the TDT system at a 6 kHz sampling rate/channel, band-pass filtered at 1-2000 Hz and averaged. The implicit time of the P1 of the
EEPs with the highest amplitude was analyzed to determine the temporal properties of EEPs. 2-D data interpolation with a 0.01 step to redefine the recorded 30-channel EEPs amplitudes, and color-coded maps, were used to locate the maximal response area and determine spatial maps.

III. RESULTS

A. In vitro Electrochemical Characteristics

1) Impedance

The impedance spectra (Fig.4a) showed that impedance magnitude was frequency dependent at frequencies lower than 10 kHz but was nearly independent of frequency at higher frequencies. The frequency dependent impedance indicated capacitive charging as the dominant current flowed. Impedance phase was also frequency dependent (Fig.4b). A phase near 0 indicated capacitive impedance at high frequencies, while a phase was near -90° indicated capacitive impedance. The impedance was 10.1 ± 0.5 kΩ at 1 kHz at room temperature. 

2) In vitro current pulse stimulation

Transient voltage responses with 0 initial bias were illustrated in Fig. 3b. Phase A was induced by the resistances of the PBS electrolyte and the electrode-electrolyte interface. The phase B voltages were caused by double-layer charging and increased with higher current pulse amplitudes. When the current pulse returned to zero, the double-layer capacitor discharged (phase C) and a relatively stable plateau at the interphase (phase D) was obtained. The voltage transients corresponding to the anodic pulses showed a similar trend to cathodic pulses, and the voltages ultimately returned to zero. The voltage drop caused by electrolyte and the electrode-electrolyte interface (Phase A) was subtracted in order to determine the voltage excursions of the electrodes. Figure 3b showed that the electrode potential at a stimulus current amplitude of 100 µA (the maximum amplitude used in the experiments) was -180 mV, a value that did not exceed the safe potential limit of -600 mV. The injected charge density for the Pt microelectrode was calculated to be 52.1 µC/cm² for a 100 µA stimulation.

B. EEPs Elicited by Suprachoroidal Stimulation

EEPs waveforms were consistently recorded from the contralateral V1 after full-row or single-electrode suprachoroidal stimulation. A typical EEPs waveform and EEPs series to varying stimulus current amplitudes were illustrated in Fig. 5a, & b, respectively. The first positive wave (P1) of the EEPs had an implicit time of 17.09 ± 1.45 ms at threshold current stimulation and this decreased as the stimulus increased. P1 amplitude versus stimulus current amplitude for single electrode stimulation showed a linear relationship such that an increase in P1 amplitude was correlated to an increase in stimulus amplitude. The polynomial linear fit for P1 and stimulus current amplitudes was shown in Fig. 5c.

1) Thresholds of EEPs for different stimulation configuration

The retina was indirectly stimulated using two configurations. The current amplitude necessary to elicit a reproducible cortical response with a full-row of electrodes was 25.55 ± 5.43 µA at 1 Hz and phase duration 0.5 ms, with corresponding charge and charge density thresholds of 12.78 ± 2.71 nC and 13.28 ± 2.82 µC/cm², respectively. The current amplitude to elicit reproducible cortical response with a single electrode was 48.57 ± 6.90 µA at 1 Hz and phase duration 0.5 ms, and the corresponding charge and charge density thresholds were 24.29 ± 3.45 nC and 25.24 ± 3.59 µC/cm², respectively.

2) Spatial spread maps of EEPs to suprachoroidal stimulation of single electrodes

A single electrode elicited EEPs from a limited area of V1, and response amplitudes (Fig. 6) varied across the cortical recording array. A decrease in stimulus amplitude caused a corresponding decrease in the spatial spread of the recorded EEPs (Fig. 7), however, the primary focus, based on the locations of the maximum P1 responses, were consistent and stable within the V1 map for a single experiment. Stimulation by different single electrodes resulted in a shift in the focus of the V1 spatial maps constructed from the maximum P1 amplitudes (Fig. 8). It was clear that the indirectly stimulated locations on the retina were spatially in correspondence with the map in visual cortex.

IV. DISCUSSION

A suprachoroidal visual prosthetic implant that indirectly stimulates the retina can potentially reduce the risk of surgical and electrical damage to the retina and has attracted the interest...
of a number of researchers [5-7, 12-14]. The purpose of a visual prosthesis is to generate a pattern of cortical phosphenes from which the patient can form a meaningful representation of a natural visual scene. An initial step in the long process of achieving this goal is to establish the response properties that can be elicited by electrode stimulation. In this study, a novel double metal-layer thin-film microelectrode array for suprachoroidal stimulation was fabricated, followed by in vitro electrochemical and in vivo response elicited evaluation.

The thin-film electrode array was designed specifically for suprachoroidal implantation and took into consideration such aspects as mechanical stiffness, ocular surgery, and microfabrication techniques. An ultra flexible thin-film electrode poses problems concerned with its insertion into the suprachoroidal space (i.e. bending and distortion), whereas a thin film with high stiffness will not be closely attached to the target, in this case the curvature of the eyeball. Consequently, the thickness must be optimized for both flexibility and stiffness. The stimulating electrodes were positioned on the surface of the array but were counter-sunk and surrounded by PI. Therefore, in order to ensure a close contact between the stimulating electrode and the tissue, it was necessary to make the outer PI coating as thin as possible (5 µm) while ensuring sufficient flexibility and stiffness of the bottom PI layer (10 µm). With respect to the metal-layer thickness, it would have been preferable that this was in the order of 100 µm for chronic electrical stimulation, however, using the present method of sputter coating to enhance Ti/Pt layer adhesion with the PI layer, would increase fabrication cost significantly if sputtering a µm-thick platinum layer. Thus, a 0.15 µm thick Pt layer was chosen along the lines of Lee et al [6].

EEPs were consistently evoked by suprachoroidal stimulation. The implicit time of the first positive EEPs wave (P1) was 17.09 ± 1.45 ms at the threshold current and decreased with increasing stimulus current amplitudes. These results were comparable with those of Nakauchi et al (15.7 ± 2 ms) [12] and similar to the implicit time recorded in cats after suprachoroidal stimulation (15.6 ± 0.94 ms [7]). EEPs amplitudes increased monotonically with increases in stimulus current amplitude in a nearly linear relationship in agreement with previous reports by Lee et al. and Shivdasani et al [6, 14]. The slope of the linear fit was similar to that reported by Lee et al [6] (0.30 µV/µA vs. 0.16 µV/µA, respectively), but was lower than that reported by Shivdasani et al (1 µV/µA [14]), which might be due to the use of full-row electrode stimulation and a large current amplitude range in their study.

The stimulus current threshold to evoke reproducible EEPs was 25.55 ± 5.43 µA for full-row electrode stimulation and 48.57 ± 6.90 µA for single electrode stimulation with a corresponding charge density threshold of 13.28 ± 2.82 µC/cm² and 25.24 ± 3.59 µC/cm², respectively. According to Liang et al. [15], a smaller electrode diameter resulted in a higher threshold charge density. The stimulating electrode diameters used by Lee et al [6] and Sakaguchi et al. [13] were 500 and 100 µm, respectively, compared to 350 µm in the present study. Thus, the charge density threshold in our experiments fall between those of the previous studies (76.47 ± 8.76 nC; 20.4 µC/cm² [6] and 66.0 ± 32.1 µA; 42.0 µC/cm² [13]). Shivdasani et al. reported that the charge thresholds for suprachoroidal stimulation were not dependent on the position of the returning electrode [14]. Cortical thresholds for single-site stimulation with a 395 µm diameter electrode with returning electrodes in the suprachoroidal space or vitreous cavity were 0.13 ± 0.02 µC, respectively. The returning electrode used in our study was arranged on the same polyimide substrate as the stimulating sites. This arrangement was a less invasive...
surgical approach and less likely to result in additional complications and retinal damage compared to inserting returning electrodes into the vitreous cavity, such as used in other studies [13, 16].

The safe charge density limit for Pt electrodes was reported to be 300 - 350 μC/cm² to avoid irreversible faradaic reactions, such as H2O electrolysis or Pt dissolution in inorganic saline [17]. We investigated the charge injection capacity for Pt microelectrodes by in vitro current pulse stimulation (100 μA, 0.5 ms pulse duration) and found that the corresponding injected charge density for the electrode was calculated to be 52.1 μC/cm² indicating a large safety margin and that higher charge injections would be possible. Adsorbed protein markedly reduces Pt dissolution [18, 19], therefore, lower dissolution rates were expected in vivo compared with in vitro electrical stimulation at equivalent charge levels. Nakauchi et al. investigated the suprachoroidal transretinal stimulation current that resulted in retinal damage in rabbits, and reported that a safe charge density limit at the electrode surface was 678 μC/cm² [16]. The largest charge density used in this study was far lower than this safe limit for either the platinum electrode or the target tissue.

The spatial characteristics of the EEPs elicited by suprachoroidal stimulation poses a key problem confronting the potential usefulness of a visual prosthesis. Shivdasani et al. [14], reported that 395 μm diameter stimulating electrodes could elicit clearly discernable responses when used individually, as a half-row, or in the full-row electrode configuration within the maximum stimulus intensity limits. In contrast, electrodes with diameters between 125 and 160 μm failed to elicit discernable responses when used in the single electrode configuration. In this study, cortical responses could be evoked at low current amplitudes and the responses were located within a limited area of the visual cortex. These results were stable with respect to decreases in stimulating current amplitude, and the focus of the responses could be altered by changing to different stimulating electrodes. Adjacent electrodes with a center-to-center distance of 750 μm when stimulated separately could result in EEPs cortical distributions that located at clearly different cortical focal points. Our results confirm the efficacy of this thin-film electrode array and provide an alternative to previously used methods and materials for suprachoroidal stimulation with a visual prosthesis.

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Xiaohong Sui received the B.E. and M.E. degrees in Microelectronics and Solid-State Electronics at Xi’an University of Technology, China in 2001 and 2004, respectively, then the Ph.D. degree in Microelectronics and Solid-State Electronics from Institute of Semiconductors, Chinese Academy of Sciences in 2007. She is currently working at Shanghai Jiao Tong University as a lecturer. Her research interests include BioMEMS, microelectrode for visual prostheses, neural interface modeling and analogue IC design.

Jingjing Sun received the B.E. degree in biomedical engineering from Shandong University, Jinan, China, in 2007. She is currently pursuing the Ph.D. degree in biomedical engineering at Shanghai Jiao Tong University, Shanghai, China. Her research interests are visual prostheses, visual prosthesis, biomedical signal processing, and electrophysiology.

Liming Li received the Ph.D. degree in biomedical engineering from Chinese Academy of Medical Sciences & Peking Union Medical College, China, in 1998. From 1998 to 2002 Dr. Li was a Postdoctoral Fellow of the Japan Society for the Promotion of Sciences (JSPS) at Kyushu Institute of Technology, Japan. She is currently an associate professor at Shanghai Jiao Tong University, China. Her research interests are in the areas of neuroprosthesis, visual electrophysiology and neuroinformatics, and biomedical signal processing.

Chuanqing Zhou received the Ph.D. degree in biomedical engineering from Shanghai Jiao Tong University in 2007. He is currently working at Shanghai Jiao Tong University as an associate professor. His research interests include ophthalmology and visual optics.

Xuejiao Luo received the B.E. degree in biomedical engineering from Beijing Institute of Technology, Beijing, China, in 2009. She is currently pursuing the M.E. degree in biomedical engineering at Shanghai Jiao Tong University. Her research interests include computer programming.

Xinyu Chai received the B.S. degree in electrical engineering from Zhengzhou University, Zhengzhou, China, in 1984, and the M.S. and Ph.D. degrees in biomedical engineering from Xi’an Jiaotong University, Xi’an, China, in 1990 and 1998, respectively. From 1999 to 2001, he was a Postdoctoral Fellow in the Department of Biomedical Engineering, Shanghai Jiao Tong University. Currently he is a professor in School of Biomedical Engineering, Shanghai Jiao Tong University. His research interests include neural engineering, artificial visual prostheses, visual psychophysics, and biomedical signal processing.

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