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Safety Assessment of Semichronic Suprachoroidal Electrical Stimulation to Rabbit Retina

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Abstract— **Confirming safety of chronic electrical stimulation is of prime importance for the practical use of visual prostheses. Here we applied electrical stimulation to eyes of freely-moving rabbits eight hour per day for one month. Examinations including fundus photo, optical coherence tomography (OCT), electrically evoked potentials (EEPs) were performed before and after one-month stimulation to detect tissue damage. No adverse effect caused by electrical stimulation was observed in electrophysiological and histological evaluation. We also found that there was no sign of morphological and electrochemical degradation of stimulating electrodes.**

I. INTRODUCTION

Visual prostheses substitutes some function of early stage of visual nervous system and send information to higher level of the system with electrical current or voltage pulses. Excessive electrical stimulation often causes tissue damage due to electrochemical irreversible reaction[1] and electrically-induced hyperactivity of neurons[2]. We have been developing a visual prostheses with suprachoroidal transretinal stimulation (STS)[3]. In STS, stimulating electrodes are placed inside sclera. Although we have tested safety of suprachoroidal electrical stimulation acutely[4], tests with longer term such as several days and months are more preferable. The FDA guideline for evaluation of retinal prostheses recommends stimulation for two days at near maximum charge limits of electrodes [5] and six month implantation without continuous activation of the device. There is no doubt that safety tests with long term electrical stimulation are beneficial to estimate damage threshold caused by electrical stimulation, but such experiments are technically difficult because keep connecting implanted electrodes with external stimulator frequently results in breakage of conductive line between electrodes and stimulator. We recently developed an experimental system which enables long-term electrical stimulation and impedance recording[6]. In this study we tried one-month daily electrical stimulation using this system to confirm whether tissue damage was caused by chronic electrical stimulation.

II. MATERIALS AND METHODS

A. Electrode Array

The electrode array consists of porous bullet-shaped platinum electrodes[7], parylene substrate with thickness of 30μm and platinum-iridium conductive line (Fig.1). Diameter and height of the electrode are 0.5mm and 0.3mm respectively. The array has two electrodes surrounded by dummy electrodes made of parylene (Fig.1C). Only one of the two electrodes was used for current pulse injection (”active electrode”). The other electrode was used only for impedance measurement (”inactive electrode”). Dummy electrodes were introduced to dissipate mechanical pressure from electrodes to the eye.

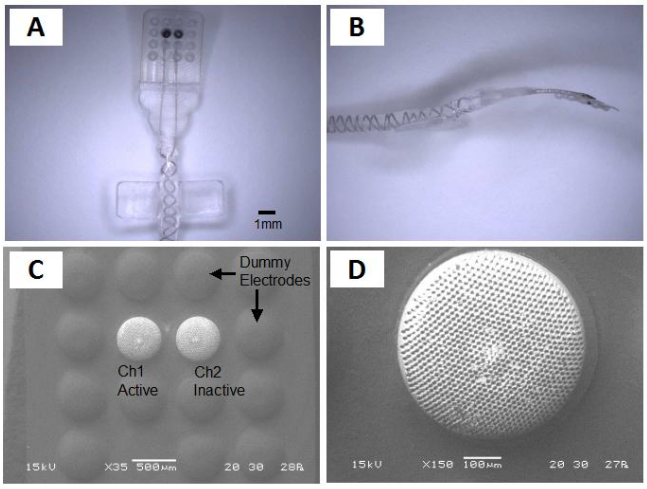

Fig. 1. (A)Top view, (B)Side view of a stimulating electrode array. The array has a curved shape (B) to fit the curvature of eyeball. (C)Two bright central electrodes of 4x4 arrangement are porous platinum electrodes[7]. Dark bumps surrounding the electrodes are dummy electrodes made of parylene. Note that electrical current pulses were applied to only one of two electrodes (Channel 1). (D) is a magnification view of stimulating electrode.

B. Implantation Surgery

Three Japanese white rabbits were used in this study. The animal was anesthetized with inhaled isoflurane. After exposing sclera by dissecting conjunctiva and inferior rectus muscle, a scleral pocket approximately 5 by 5 mm was formed with a crescent knife at lower-temporal area 9 mm from the corneal limbus. The electrode array was inserted into the scleral pocket, then cable was sutured onto sclera. The return electrode, 0.5mm-diameter 3mm-long platinum bar, was implanted at upper nasal area 3mm from the corneal limbus. A recording electrode made of 1.4mm-diameter platinum ball was placed onto visual cortex (8mm posterior to lambda cranial suture and 6.5mm lateral to the midline) contralateral to the implanted eye. A reference electrode for EEP measurement, a 2mm-diameter 6mm-long stainless

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screw, was implanted at blegma. All in vivo experiments were conducted in accordance with the Association for Research in Vision and Ophthalmology statement for the use of animals in ophthalmic and vision research, and institutional guidelines for the care and use of laboratory animals.

C. Electrical Stimulation

After two-week recovery period, an external stimulator was connected to implanted electrodes. A slip ring was mounted on the roof of animal cage so that animals could move freely in the cage without breaking conductive lines by their rotational movement. Cathodic-first current pulses with 1.5mA amplitude and 0.5ms width at repetition frequency of 50Hz were applied to one of two electrodes (Channel 1, Fig.1C) for eight hour per day for one month.

D. Ophthalmic and Electrochemical examination

A series of examination was performed before and after one-month stimulation. EEPs were recorded with our custom-made stimulator and commercially available amplifier (MEG-6116, Nihon Kohden). Cathodic-first 1mA-amplitude 1ms-width current pulses were applied to evoke EEPs. Electrical stimulation was repeated 1000 times at 2Hz and averaged waveform was recorded with software (EplyzerII, KISSEI COMTEC) after bandpass filtering between 1.5Hz and 1kHz. Then fundus photo (RetCam, Massie Research Laboratories) and OCT (RS-3000, Nidek) was also recorded.

E. Charge injection capacity *in vitro* and *in vivo*

Before implantation, charge injection capacity was measured in phosphate-buffered saline at room temperature. The charge injection capacity is defined as the maximum charge density without deviating water window of platinum (-0.6 to +0.8V vs Ag/AgCl [8]) during charge injection. Cathodic-First 500μs-duration current pulse with 30Hz were employed for measurements. Electrode potential during pulsing was recorded with custom-made amplifier and oscilloscope (DL750, Yokogawa). Cyclic voltammogram was then obtained with potentiostat (PGSTAT12, Metrohm Autolab). The same measurements were performed again after explantation of stimulating electrode array.

During stimulation period, charge injection capacity was measured *in vivo*. Detailed measurement procedure was reported elsewhere[9]. In brief, an Ag/AgCl electrode was electrically connected to rabbit via needle and saline-filled tube. *In vivo* electrode potential was measured with reference to the Ag/AgCl electrode. Measurement was performed before and after one-month stimulation period.

F. Histological Evaluation

After one-month electrical stimulation, stimulated eyes were enucleated and fixated in the mixture of 1.5% glutaraldehyde and 3% formalin. After dehydration and embedding with paraffin, eyes were cut into sections and stained with hematoxylin and eosin. Tissue samples were examined with optical microscope (ECLIPSE E1000, Nikon).

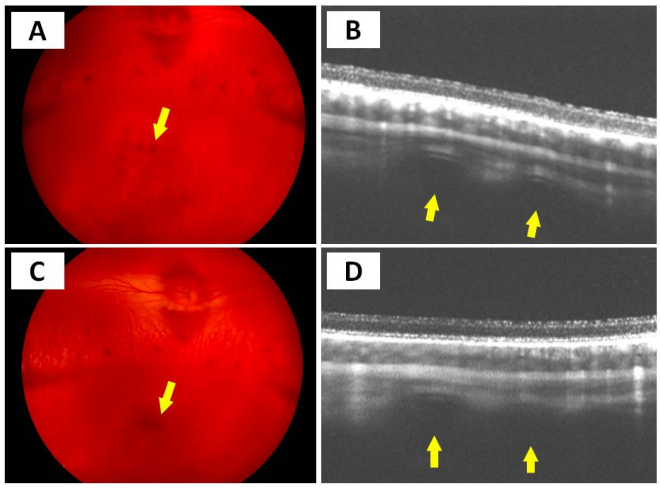

Fig. 2. Fundus photograph (left column) and OCT image (right column) of a rabbit eye before stimulation (A,B) and after one-month stimulation (C,D). Electrodes are indicated by yellow arrows.

G. Examination of explanted electrodes

Explanted stimulating electrodes were examined with scanning electron microscope (JSM-5600LV, JEOL) and compared with images obtained before implantation to detect any sign of electrode degradation.

III. RESULTS

Stimulating electrode array was successfully visualized both in fundus photo and OCT (Fig.2). Suprachoroidal electrodes are difficult to visualize in normal fundus photo, but reducing illumination significantly enhanced visibility of electrodes. Residual scleral thickness (distance between the tip of stimulating electrode and choroid) ranged 100 μm to 300μm in OCT images. In one animal, fundus observation was difficult due to corneal opacity caused by implantation surgery, but it finally recovered after one-month stimulation period. No sign of choroidal ischemia due to mechanical pressure from electrodes were observed in OCT images.

EEPs were recorded at time points both pre-stimulation and post-stimulation (Fig.3). No significant differences of latencies and amplitudes were detected between waveforms before and after one-month stimulation. Similarly, no differences were observed between active electrode (Channel 1, Fig.3 left) and inactive electrode (Channel 2, right).

Histological samples exhibited some amount of cellular concentration at the interface between electrode and scleral tissue (Fig.4 E,F). This suggests inflammatory response did occur, but the thickness of inflammatory tissue was smaller than 50μm and did not significantly affected the distance between stimulating electrode and retinal neurons. No differences were observed between samples of active electrodes and that of inactive electrode, suggesting that electrical stimulation did not play a major role for tissue response.

Charge injection capacity measured in phosphate-buffered saline (PBS) before implantation was higher than 0.7mC/cm^2

in average (Fig.5). This is relatively high for platinum electrode[8]. The high charge injection capacity is presumably explained by the porosity of stimulating electrodes[7].

In vivo charge injection capacity was much lower than that *in vitro* (Fig.5). There were no statistically significant differences of charge injection capacity between active electrodes (channel 1) and inactive electrodes (channel 2).

Charge injection capacity was again measured in PBS after explantation. No statistically significant differences were detected between charge injection capacities before implantation and after explantation for active and inactive electrodes. Similarly, the shape of cyclic voltammogram did not differ before and after implantation (Fig.6).

Figure 7 shows morphological comparison of an active electrode (Channel 1) before and after one-month *in vivo* simulation. No sign of electrode degradation was observed.

IV. DISCUSSION

In semichronic clinical trial of suprachoroidal prosthesis, threshold charge per pulse was ranged from 0.175 to 0.45 μC/phase with repetition frequency of 20Hz[10]. Charge per phase employed in this study was 0.75μC/phase with 50Hz repetition frequency. The pulse parameters for this study was determined so that the strength of stimulation significantly exceeds the maximum charge necessary in clinical trials. Even though we employed such a high-strength stimulation, we did not detect any proof of tissue damage in fundus photo, OCT image and histological analysis. These results suggests that electrical stimulation up to 0.75μC/phase with 50Hz repetition frequency is safe. In addition, charge injection capacities and cyclic voltammogram did not significantly differ between before and after one-month stimulation. Such electrochemical properties also did not differ between active electrodes (channel 1) and inactive electrodes (channel 2). Therefore we concluded that degradation of electrodes such as corrosion did not occur in this study. This conclusion was farther supported by scanning electron microscopy (Fig.7).

Charge injection capacity significantly differ between *in vitro* and *in vivo*(Fig.5). This is consistent with previous reports[11][12]. The reason for the difference is not clear, but phenomena such as biomolecule adhesion to electrodes, electrode encapsulation and low availability of counterion are likely to play a dominant role for the difference[12].

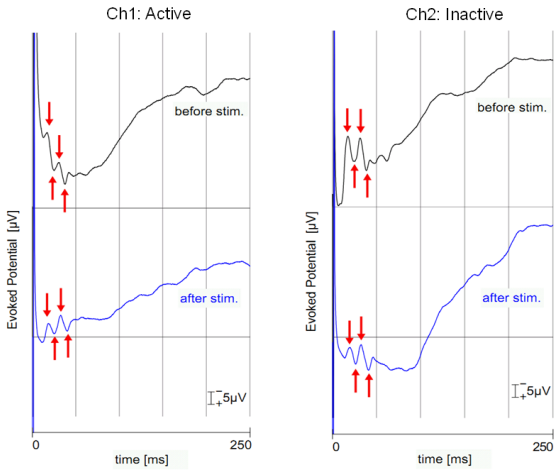

Fig. 3. EEPs obtained with electrical stimulation from active electrode (left) and inactive electrode (right). Black lines and blue lines indicates EEPs before and after one-month stimulation respectively.

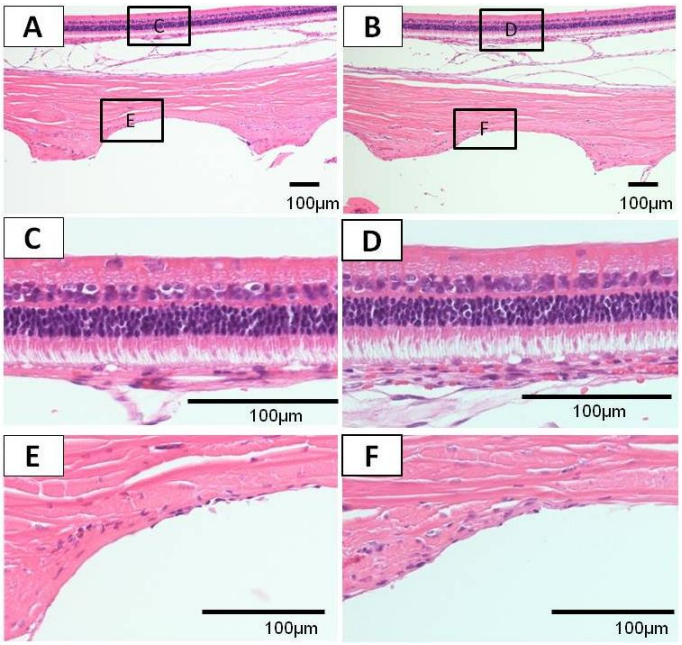

Fig. 4. Cross section of the eye stained with hematoxylin and eosine. (A)Tissue around an active electrode (Channel 1). (B) Tissue around an inactive electrode (Channel 2). (C) Magnification view of the retina of (A). (D) Magnification view of the retina of (B). (E) Magnification view of interfacial area between electrode and sclera of (A). (F) Magnification view of interfacial area between electrode and sclera of (B).

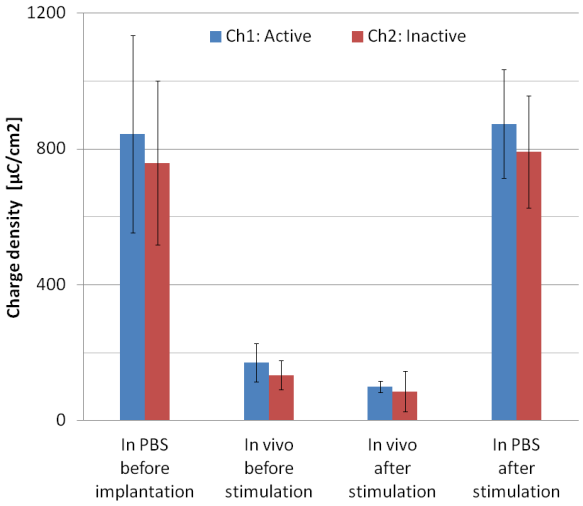

Fig. 5. Charge injection capacities measured *in vitro* (in phosphate-buffered saline (PBS)) and *in vivo*. Bar length and errorbar represents mean $\pm$ S.D. (n=3).

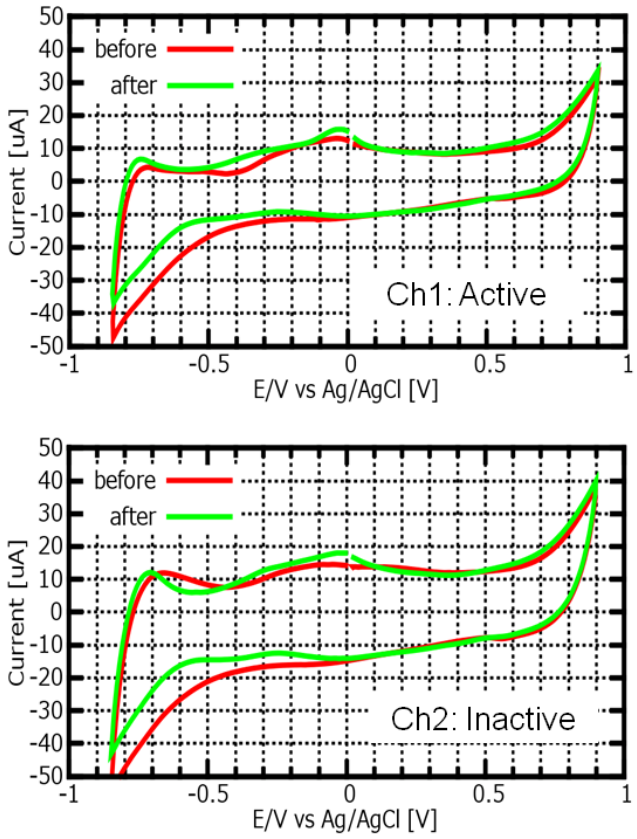

Fig. 6. Cyclic voltammogram of an active electrode (top) and an inactive electrode (bottom). The red lines indicate data before implantation. The green lines corresponds data after explantation. All measurements were performed in phosphate-buffered saline at room temperature.

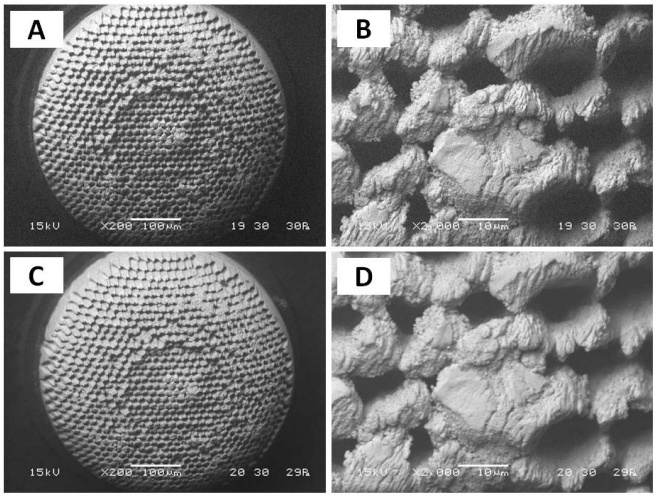

Fig. 7. Scanning electron microscopy of an active electrode (Channel 1) before implantation (A,B) and after explantation (C,D).

V. CONCLUSION

One-month daily suprachoroidal electrical stimulation up to 0.75μC/phase with 50Hz repetition frequency using porous platinum electrodes was suggested to be safe. The electrodes did not degrade after one-month stimulation. We will try similar experiments with longer stimulation period in the next step.

REFERENCES

[1] S. B. Brummer and M. J. Turner. Electrochemical considerations for safe electrical stimulation of the nervous system with platinum electrodes. *IEEE Trans. Biomed. Eng.*, Vol. 24, No. 1, pp. 59–63, Jan 1977.

[2] D. B. McCreery, W. F. Agnew, T. G. Yuen, and L. A. Bullara. Comparison of neural damage induced by electrical stimulation with faradaic and capacitor electrodes. *Ann Biomed Eng*, Vol. 16, No. 5, pp. 463–481, 1988.

[3] H. Kanda, T. Morimoto, T. Fujikado, Y. Tano, Y. Fukuda, and H. Sawai. Electrophysiological studies of the feasibility of suprachoroidal-transretinal stimulation for artificial vision in normal and rcs rats. *Invest Ophthalmol Vis Sci*, Vol. 45, No. 2, pp. 560–566, Feb 2004.

[4] K. Nakauchi, T. Fujikado, H. Kanda, S. Kusaka, M. Ozawa, H. Sakaguchi, Y. Ikuno, M. Kamei, and Y. Tano. Threshold suprachoroidal-transretinal stimulation current resulting in retinal damage in rabbits. *J Neural Eng*, Vol. 4, No. 1, pp. S50–S57, Mar 2007.

[5] Draft guidance for industry and fda staff: Investigational device exemption (ide) guidance for retinal prostheses. Available at http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm135321.htm, 2009.

[6] H. Tashiro, Y. Terasawa, M. Shinomiya, M. Ozawa, T. Noda, and J. Ohta. Long-term suprachoroidal-transretinal stimulation by the bullet-shaped platinum electrodes in normal rabbits. In *ARVO E-abstracts*, p. 4947, 2011.

[7] Y. Terasawa, H. Tashiro, K. Osawa, M. Ozawa, T. Noda, J. Ohta, and T. Fujikado. Porous platinum electrodes for retinal prostheses. In *IOVS*, No. 53, pp. E–Abstract 5538, 2012.

[8] S. F. Cogan. Neural stimulation and recording electrodes. *Annu Rev Biomed Eng*, Vol. 10, pp. 275–309, 2008.

[9] H. Tashiro, Y. Terasawa, K. Osawa, M. Ozawa, T. Noda, J. Ohta, and T. Fujikado. In vivo characterization of electrochemically-treated platinum bulk electrodes for retinal prostheses. *ARVO E-abstract*, 2012.

[10] T. Fujikado, M. Kamei, H. Sakaguchi, H. Kanda, T. Morimoto, Y. Ikuno, K. Nishida, H. Kishima, T. Maruo, K. Konoma, M. Ozawa, and K. Nishida. Testing of semichronically implanted retinal prosthesis by suprachoroidal-transretinal stimulation in patients with retinitis pigmentosa. *Invest Ophthalmol Vis Sci*, Vol. 52, No. 7, pp. 4726–4733, Jun 2011.

[11] Z. Hu, P. R. Troyk, T. P. Brawn, D. Margoliash, and S. F. Cogan. In vitro and in vivo charge capacity of airof microelectrodes. *Conf Proc IEEE Eng Med Biol Soc*, Vol. 1, pp. 886–889, 2006.

[12] S. F. Cogan. In vivo and in vitro differences in the charge-injection and electrochemical properties of iridium oxide electrodes. *Conf Proc IEEE Eng Med Biol Soc*, Vol. 1, pp. 882–885, 2006.