An electrically resistive sheet of glial cells for amplifying signals of neuronal extracellular recordings

R. Matsumura,¹ H. Yamamoto,^{2,a)} M. Niwano,³ and A. Hirano-Iwata¹

¹Graduate School of Biomedical Engineering, Tohoku University, 6-6 Aramaki-aza Aoba, Aoba-ku, Sendai 980-8579, Japan

²Frontier Research Institute for Interdisciplinary Sciences, Tohoku University, 6-3 Aramaki-aza Aoba, Aoba-ku, Sendai 980-8578, Japan

³Research Institute of Electrical Communication, Tohoku University, 2-1-1 Katahira, Aoba-ku, Sendai 980-8577, Japan

(Received 2 November 2015; accepted 24 December 2015; published online 11 January 2016)

Electrical signals of neuronal cells can be recorded non-invasively and with a high degree of temporal resolution using multielectrode arrays (MEAs). However, signals that are recorded with these devices are small, usually 0.01%–0.1% of intracellular recordings. Here, we show that the amplitude of neuronal signals recorded with MEA devices can be amplified by covering neuronal networks with an electrically resistive sheet. The resistive sheet used in this study is a monolayer of glial cells, supportive cells in the brain. The glial cells were grown on a collagen-gel film that is permeable to oxygen and other nutrients. The impedance of the glial sheet was measured by electrochemical impedance spectroscopy, and equivalent circuit simulations were performed to theoretically investigate the effect of covering the neurons with such a resistive sheet. Finally, the effect of the resistive glial sheet was confirmed experimentally, showing a 6-fold increase in neuronal signals. This technique feasibly amplifies signals of MEA recordings. © 2016 AIP Publishing LLC.

[<http://dx.doi.org/10.1063/1.4939629>]

Microelectrode array (MEA) technology is widely used to record trains of action potentials from cultured neurons, cardiomyocytes, or brain slice preparations.^{1–4} The major advantage of this method lies in its high temporal resolution (>10 kHz) and non-invasiveness. MEA recordings have been used both in fundamental studies, e.g., to analyse activity patterns of cultured neuronal networks,^{5,6} and in pharmacological research, for screening lead compounds *in vitro*.^{7,8} However, small signals that can be detected through extracellularly positioned microelectrodes inhibit MEAs from being used in further applications such as studies of subthreshold activity. The amplitude of extracellularly recorded signals is usually in the order of ten to a hundred microvolts, which is 3–4 orders of magnitude lower than the intracellular change of membrane potential in an action potential (~100 mV).

Various attempts have been made to overcome this issue. One approach involves increasing the seal resistance between the cell and the electrode. This can be achieved by using nanostructured electrodes instead of planar electrodes that become engulfed by overlying cells.^{1,9} A second approach is to decrease the electrode impedance. To record activity from single cells, the use of a smaller electrode is preferable, but this comes at the cost of increased electrode impedance, which decreases the signal. Wolfrum *et al.* overcame this dilemma by creating micropores that interface the cells to larger electrodes.^{10,11} A third approach involves decreasing the membrane impedance at the cell-electrode junction. In practice, this involves transiently rupturing the

cell membrane by applying a zap voltage to a nanostructured electrode, thus allowing the electrodes to enter the cell and perform pseudo-intracellular recording until the ruptured membrane reorganizes.^{1,12,13}

In this letter, we propose an alternative approach to increase the neuron-electrode seal for amplifying signals in extracellular recordings. This is achieved by covering neurons with an electrically resistive sheet. The resistive sheet used in this study was made of glial cells cultured in a monolayer on a collagen-gel film. Glial cells are supportive cells in the central nervous system. More specifically, the class of glial cells we used was astrocytes, which take up and release ions and molecules to maintain their extracellular concentration or to supply them to neurons. Concurrently, the cell membrane is a biological insulating layer. A sheet composed of glial cells thus balances the competing goals of electrical resistance and nutrient permeability, making them much more attractive than other insulating materials. We experimentally and theoretically examined how a neuronal signal is amplified by covering cultured neurons with the resistive glial sheet.

The collagen-gel film was prepared following a previously reported protocol.¹⁴ Briefly, a nylon membrane (Hybond-N⁺, Amersham) in the form of a ring was prepared using a paper punch (inner diameter: 9.5 mm and outer diameter: 15.9 mm). The nylon ring was sterilized in 70% ethanol and then placed in a 12 well plate. A type-I collagen solution (5 mg ml⁻¹; AteloCell IAC-50, Koken) was mixed 1:1 with serum-containing medium [Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS), 5% horse serum, 20 mM HEPES, and 1% penicillin/streptomycin] on ice, and the mixture was immediately poured into the well plate. After

^{a)}Author to whom correspondence should be addressed. Electronic mail: h-yamamoto@bme.tohoku.ac.jp. Tel.: +81-22-795-4866. Fax: +81-22-795-4863.

FIG. 1. (a) Schematic illustration (*left*) and photographs (*middle, right*) of a glial sheet formed on a collagen-gel film. (b) Fluorescence micrograph of the glial cells. The cells were stained with GFAP (an astrocyte marker, green) and DAPI (blue). Scale bar: 50 μm . (c) Phase-contrast micrograph of the glial cells. Scale bar: 100 μm .

a 2 h incubation at 37°C for gelation, the collagen gel was cooled in a 4°C refrigerator overnight. On the following day, the gel was transferred to a clean bench and dried under a flow of filtered air. Prior to cell culture, the collagen gel was rehydrated in glial-differentiation medium (described below) and immersed in a poly-D-lysine (PDL) solution [50 $\mu\text{g ml}^{-1}$ in phosphate buffered saline (PBS)] and then in a laminin solution (10 $\mu\text{g ml}^{-1}$ in

PBS), each was kept overnight, to promote cell adhesion and growth [Fig. 1(a)].

The resistive glial sheet was fabricated by culturing the glial cells on the collagen-gel film for a minimum of 10 days. The glial cells were obtained by differentiating primary mouse neural stem cells in glial-differentiation medium (DMEM/F12 containing N-2 supplement, 10% FBS, and 1% penicillin/streptomycin) that contains 10% serum to direct differentiation to astrocytes.^{15,16} By immunostaining the glial sheet with an astrocyte marker, the glial fibrillary acidic protein (GFAP; rabbit polyclonal; Synaptic Systems 173–002), we confirmed that all cells had successfully differentiated into astrocytes [Fig. 1(b)]. Note that GFAP stains only ~15% of the total volume of astrocytes¹⁷ and that the cells grew in a well-packed monolayer, possibly forming connexin-mediated gap junctions between cells,¹⁸ as evidenced by a phase-contrast micrograph [Fig. 1(c)].

Electrochemical impedance spectroscopy (EIS; VersaSTAT 3.0, Princeton Applied Research) was then used to evaluate the electrical properties of the glial sheet. A glial sheet was placed upside-down on a multielectrode array (MED-P210A, AlphaMED Scientific), and a block of polydimethylsiloxane (PDMS; diameter: 6.5 mm and weight: ~0.4 g) was placed on the top to prevent the sheet from floating [Fig. 2(a)]. Measurements were performed in the culture medium using a three electrode configuration. Pt-black microelectrodes (20 $\times$ 20 μm^2) were used as the working electrode, an internal large Pt-black electrode (200 $\times$ 200 μm^2) as the reference

FIG. 2. (a) Photograph of a resistive glial sheet mounted on an MEA device. RE, reference electrode and CE, counter electrode. Size of the MEA device was 5 $\times$ 5 cm^2 . (b) Average impedance of bare electrodes, electrodes covered with a collagen-gel film, and electrodes covered with a resistive glial sheet, i.e., a collagen-gel film with glial cells. Error bars: s.e.m. (c) Cole-Cole plots of an electrode without (black) and with (red) a resistive glial sheet. The green curve shows the best fit of the red curve. (d) Equivalent circuit model used for curve fitting. The model consisted of a solution contribution (R_{sol}), an electrode contribution (R_e , C_e), and a resistive glial sheet contribution (R_{gs} , C_{gs}). The values for the best fit are shown in the box.

electrode, and a Pt wire as the counter electrode. The amplitude of the applied signal was 10 mV, and the frequency was scanned from 1 kHz to 1 MHz. Impedance spectra were analyzed on ZView (Scribner Associates).

As summarized in Fig. 2(b), the electrode impedance at 16 kHz (Ref. 19) was increased 3.2-fold when the electrode was covered with the resistive glial sheet. In a control experiment using a collagen-gel film without glial cells, the increase in impedance was only 1.4-fold. This confirms that the resistive effect can be attributed to the glial cells and not to the collagen-gel film or the PDMS block. The factor that limited the rise in electrode impedance to 3.2-fold is most likely the small seal resistance between the glial sheet and the electrode, since the glial cells did not adhere to the electrodes but were only physically attached to them. A Cole-Cole plot of the electrode before and after coverage is shown in Fig. 2(c). The measured spectrum was fit to a RC parallel circuit model to derive the parameters for the electrode and the glial sheet [Fig. 2(d)].

Next, circuit simulations were conducted to theoretically investigate the effect of covering neuronal cultures with the resistive glial sheet. Simulations were performed using TINA-TI (Texas Instruments). The passive analog circuit model and its correspondence with the cell-electrode interface is illustrated in Fig. 3(a). The circuit model was prepared based on a previous report,¹ and the resistive glial sheet was inserted serially next to the non-junctional impedance and the voltage source. Values for the junctional and non-junctional membrane impedances were obtained from the literature with appropriate modifications,¹ assuming a membrane resistivity of $10\text{ k}\Omega\text{ cm}^2$, a specific capacitance of $1\text{ }\mu\text{F cm}^{-2}$, a junctional area of $1 \times 10^{-6}\text{ cm}^2$, and a non-junctional area of $1 \times 10^{-4}\text{ cm}^2$. The parameters obtained from EIS measurements [Fig. 2(d)] were used for the electrode and the glial sheet impedance. A depolarizing pulse mimicking a neuronal action potential (100 mV and 1 ms) was applied to the voltage source, and the amplitude of the output was measured. By inserting the glial sheet impedance, the voltage output was found to increase by 4.7 times [Fig. 3(b)].

To experimentally confirm this result, we next investigated the effect of covering neuronal cultures with the resistive glial sheet on the signal amplitude of extracellular recordings. For this, we recorded the spontaneous activity of a neuronal network grown on a MEA and compared the amplitude of the signals before and after coverage. Primary neurons were obtained from embryonic rat hippocampi, plated on a PDL-coated MEA chip at a density of $1.4 \times 10^5\text{ cells cm}^{-2}$, and cultured in Neurobasal medium (Neurobasal containing 2% B-27 supplement and 1% GlutaMAX-I) for 2 weeks. Signal recordings were performed using the NeuroLog System (NL104 AC preamplifier and NL125 filters, Digitimer). Signals were sampled at a rate of 10 kHz using the PowerLab 4/30 AD converter and the Lab Chart software program (ADInstruments).

Figs. 4(a) and 4(b) show the recorded signals before and after covering the neurons with the resistive glial sheet, respectively. A large increase in the spike amplitude could be confirmed. The spikes were completely abolished when the Na-channel blocker tetrodotoxin ($5\text{ }\mu\text{M}$) was added, providing evidence that they are derived from neuronal activity [Fig. 4(c)]. Quantitative evaluation of signal amplitudes

FIG. 3. (a) Schematic illustration depicting the relationship between an equivalent circuit model using analog passive elements and an actual experiment. A neuron covered with the resistive glial sheet resides on a working electrode of MEA. R_{gs} : glial sheet resistance ($41\text{ k}\Omega$), C_{gs} : glial sheet capacitance (114 pF), R_{njm} : non-junctional membrane resistance ($100\text{ M}\Omega$), C_{njm} : non-junctional membrane capacitance (100 pF), R_{jm} : junctional membrane resistance ($10\text{ G}\Omega$), C_{jm} : junctional membrane capacitance (1 pF), R_{seal} : neuron-electrode seal resistance ($1\text{ M}\Omega$), R_e : electrode resistance ($6.7\text{ k}\Omega$), and C_e : electrode capacitance (49 pF). A square pulse of 100 mV was applied to the signal source for 1 ms, and the output that passed through the voltage follower circuit and low-pass filter was monitored. (b) The calculated amplitude of the output signal without and with the glial sheet impedance. The values are presented after normalization with the signal amplitude of the circuit without glial sheet impedance.

revealed that the average amplitude increased 6 times, from $10.3\text{ }\mu\text{V}$ to $57.5\text{ }\mu\text{V}$ ($n = 50$ spikes), as the result of covering the neurons with the resistive glial sheet [Fig. 4(d)]. The rather small amplitude of the signals is primarily caused by the use of a home-made device holder [Fig. 2(a)], which resulted in stray resistance being added between the electrode and the preamplifier. Covering the neurons with the glial sheet also increased background noise, which is most probably due to the physiological activity of the glial cells. This effect, however, was minor compared with the signal amplification that arose from the increase in neuron-electrode seal. An estimation of the signal-to-noise ratio revealed that it increased from 1.9 to 9.3 by the glial sheet treatment [Fig. 4(d)].

Finally, we tested whether the glial sheet could be detached from the neuronal culture after the recordings. For this, we covered cultured neurons with a glial sheet for 10 min, removed the glial sheet, and compared the micrographs before and after the process. Neurons and their neurites remained morphologically intact [Fig. 5(a)], although in some cells, cell bodies were abducted by the glial sheet [Fig. 5(b)]. This shows that neuronal cultures can be sustained for further use, even after performing a measurement with the resistive glial sheet.

FIG. 4. Raw traces of MEA recordings from an electrode before (a) and after (b) the application of the resistive glial sheet. (c) The spikes are diminished after adding tetrodotoxin. (d) Average amplitude of neural signals (*left*) and signal-to-noise ratio (*right*) before and after application of the resistive glial sheet ($n = 50$ spikes). Error bars: s.e.m.

For experiments where damage to cultured neurons needs to be minimized, several approaches could be considered to reduce the adhesion of glial sheet to neurons. Previous studies in the area of molecular biology revealed

that neuron-glia adhesion is mediated by the binding of neuronal Thy-1 and glial integrin $\alpha_v\beta_3$ and that this binding occurs in less than 20 min after cell-cell contact.²⁰ The Thy-1/integrin interaction can be blocked by the RGD peptide,²¹ and hence adding the peptide to the bath solution can decrease the extent of neuronal abduction by the glia sheet. Alternatively, pre-treating the glial cell membrane with an anti-fouling agent such as polyethylene glycol or polyvinyl alcohol could also have positive effects in reducing the extent of neuron-glia adhesion.²²

Importantly, the method proposed here is fully compatible with other approaches for signal amplification, e.g., using nanostructured electrodes.^{1,4,9–13} These approaches have already been successful in recording large spike signals. For example, Czeschik *et al.* used nanocavity electrodes and reported recordings of up to ~ 3 mV from cardiomyocytes.¹¹ The signal amplitude would be expected to be further amplified by incorporating the resistive sheet covering method.

In the case of implanted electrodes, the insulating effect of glial cells imposes a negative effect. Astrocytes and microglia form an encapsulation layer known as a “glial scar” around the probe, which increases electrode impedance and decreases its performance over time.^{23,24} Current approaches to the issue include optimizing the flexibility and size of the probe or coating the probe surface with bio-compatible molecules to minimize the immune response.^{24,25} Our findings suggest an alternative strategy for overcoming this issue—the immune response can act positively, provided the recording configuration can be changed so that the glial cells sheath the electrodes together with the neural tissue of interest.

FIG. 5. Phase-contrast images of primary neurons cultured on a micropatterned coverslip. Consecutive images were taken before application of the resistive glial sheet (left panels) and after its application and removal (right panels). (a) Cells remained intact after the process. (b) Cell bodies of some neurons (indicated with black arrows) were removed as the result of the removal of the resistive glial sheet. Scale bar: 100 μm .

In conclusion, we demonstrated herein that the signal amplitude of neuronal extracellular recordings can be amplified by 6 times by covering cultured neurons with a resistive glial sheet. The signal amplification effect was supported by equivalent circuit simulation with impedance values evaluated by EIS. The method is feasible and can be used in combination with, e.g., nanostructured electrodes. These technologies have the potential to expand the applications of MEA devices in fundamental studies and in biomedical applications.

The work was supported by the Research Fellowships for Young Scientists (No. 15J03545) and the Grant-in-Aid for Young Scientists (B) (No. 15K17449) from the Japan Society for the Promotion of Science, by the CREST Program from the Japan Science and Technology Agency, and by a research grant from the Asahi Glass Foundation. The authors wish to thank Professor Shutaro Katsurabayashi (Fukuoka University) for providing reagents and Mr. Hidesato Takaoki (Tohoku University) for technical support.

¹M. E. Spira and A. Hai, *Nat. Nanotechnol.* **8**, 83 (2013).

²R. Kim, S. Joo, H. Jung, N. Hong, and Y. Nam, *Biomed. Eng. Lett.* **4**, 129 (2014).

³M. E. J. Obien, K. Deligkaris, T. Bullmann, D. J. Bakkum, and U. Frey, *Front. Neurosci.* **8**, 423 (2015).

⁴M. R. Angle, B. Cui, and N. A. Melosh, *Curr. Opin. Neurobiol.* **32**, 132 (2015).

⁵R. Segev, Y. Shapira, M. Benveniste, and E. Ben-Jacob, *Phys. Rev. E* **64**, 011920 (2001).

⁶N. Friedman, S. Ito, B. A. W. Brinkman, M. Shimono, R. E. L. DeVille, K. A. Dahmen, J. M. Beggs, and T. C. Butler, *Phys. Rev. Lett.* **108**, 208102 (2012).

⁷A. Novellino, B. Scelfo, T. Palosaari, A. Price, T. Sobanski, T. J. Shafer, A. F. M. Johnstone, G. W. Gross, A. Gramowski, O. Schroeder, K. Jügelt,

M. Chiappalone, F. Benfenati, S. Martinoia, M. T. Tedesco, E. Defranchi, P. D'Angelo, and M. Whelan, *Front. Neuroeng.* **4**, 4 (2011).

⁸K. Harris, M. Aylott, Y. Cui, J. B. Louttit, N. C. McMahon, and A. Sridhar, *Toxicol. Sci.* **134**, 412 (2013).

⁹F. Santoro, S. Dasgupta, J. Schnitker, T. Auth, E. Neumann, G. Panaitov, G. Gompper, and A. Offenhäusser, *ACS Nano* **8**, 6713 (2014).

¹⁰B. Hofmann, E. Katelhon, M. Schottdorf, A. Offenhäusser, and B. Wolfrum, *Lab Chip* **11**, 1054 (2011).

¹¹A. Czeschik, A. Offenhäusser, and B. Wolfrum, *Phys. Status Solidi A* **211**, 1462 (2014).

¹²J. T. Robinson, M. Jorgolli, A. K. Shalek, M.-H. Yoon, R. S. Gertner, and H. Park, *Nat. Nanotechnol.* **7**, 180 (2012).

¹³C. Xie, Z. Lin, L. Hanson, Y. Cui, and B. Cui, *Nat. Nanotechnol.* **7**, 185 (2012).

¹⁴T. Takezawa, K. Ozaki, A. Nitani, C. Takabayashi, and T. Shimo-oka, *Cell Transplant.* **13**, 463 (2004).

¹⁵J. Ray and F. H. Gage, *Mol. Cell. Neurosci.* **31**, 560 (2006).

¹⁶A. M. Haidet-Phillips, M. E. Hester, C. J. Miranda, K. Meyer, L. Braun, A. Frakes, S.-W. Song, S. Likhite, M. J. Murtha, K. D. Foust, M. Rao, A. Eagle, A. Kammesheidt, A. Christensen, J. R. Mendell, A. H. M. Burghes, and B. K. Kaspar, *Nat. Biotechnol.* **29**, 824 (2011).

¹⁷E. A. Bushong, M. E. Martone, Y. Z. Jones, and M. H. Ellisman, *J. Neurosci.* **22**, 183 (2002).

¹⁸R. Dermietzel, E. L. Hertzberg, J. A. Kessler, and D. C. Spray, *J. Neurosci.* **11**, 1421 (1991).

¹⁹Y.-S. Hsiao, C.-W. Kuo, and P. Chen, *Adv. Funct. Mater.* **23**, 4649 (2013).

²⁰M. Kong, N. Muñoz, A. Valdivia, A. Alvarez, R. Herrera-Molina, A. Cárdenas, P. Schneider, K. Burridge, A. F. Quest, and L. Leyton, *Biochim. Biophys. Acta* **1833**, 1409 (2013).

²¹L. Leyton, P. Schneider, C. V. Labra, C. Rüegg, C. A. Hetz, A. F. G. Quest, and C. Bron, *Curr. Biol.* **11**, 1028 (2001).

²²Y. Teramura, Y. Kaneda, and H. Iwata, *Biomaterials* **28**, 4818 (2007).

²³V. S. Polikov, P. A. Tresco, and W. M. Reichert, *J. Neurosci. Methods* **148**, 1 (2005).

²⁴J. P. Frampton, M. R. Hynd, M. L. Shuler, and W. Shain, *Ann. Biomed. Eng.* **38**, 1031 (2010).

²⁵T. D. Y. Kozai, A. S. Jaquins-Gerstl, A. L. Vazquez, A. C. Michael, and X. T. Cui, *ACS Chem. Neurosci.* **6**, 48 (2015).