Ultra-Low Cost, Facile Fabrication of Transparent Neural Electrode Array for Electrocorticography with Photoelectric Artifact-Free Optogenetics

Young Uk Cho, Ju Young Lee, Ui-Jin Jeong, Sang Hoon Park, Se Lin Lim, Kyung Yeun Kim, Je Wu Jang, Jong Ho Park, Hyun Woo Kim, Hyogeun Shin, Hojeong Jeon, Young Mee Jung, Il-Joo Cho,* and Ki Jun Yu*

Transparent implantable devices have received significant attention in neuroscience and biomedical engineering by combining neural recording and optical modalities. Opaque, metal-based electrode arrays for electrophysiology block optical imaging and cause photoelectric artifacts, making them difficult to integrate with optogenetics. Here, a photoelectric artifact-free, highly conductive, and transparent poly(3,4-ethylenedioxythiophene) polystyrene sulfonate (PEDOT:PSS) electrode array is introduced as promising neural implants. The technology which is developed in this work provides transparent neural interfaces through low-cost, ultra-facile method compared with other transparent materials being applied to implantable tools. The device exhibits superior optical, mechanical, and electrical characteristics to other studies, thanks to a simple ethylene glycol immersing process. The device performance is highlighted by comparing its light stimulation efficiency and photoelectric artifact extent with conventional thin gold electrodes both in vitro and in vivo. This platform can assemble transparent neural interfaces much more efficiently than any other material candidates and thus has many potential applications.

1. Introduction

Unraveling complex neural circuits through various modalities is of great value in identifying neurodegenerative diseases in patients with chronic pain.[3] There are mainly two modalities of identifying diagnosing neurological disorder—electrophysiological approaches and optical approaches. Electrophysiological recording method has been able to cover the disadvantages of relatively low time-resolution of structural brain imaging, such as magnetic resonance imaging or positron emission tomography, by real-time biological signals readout property.[3] However, beyond passive recording, studying comprehensive neural activity and developing treatments require neuromodulation methods, such as electrical stimulation,[3] drug delivery,[4]...
and optogenetics. Optogenetics has attracted significant attention thanks to its sophisticated stimulation effects. It is easy to adjust stimuli intensity through cell-type-specific selectivity. Combined with electrophysiological recordings, optogenetics can help explore new neuroscience areas by allowing real-time identification of neuronal activity changes. However, combining conventional metal-based electrode arrays with optical modalities causes serious problems. First, opaque metal electrode arrays block the field of view in biological tissue, preventing simultaneous optical imaging and electrophysiological signal recording. There were several approaches to address this problem using nano-network, nano-mesh or circular structure of opaque metal as examples. These methods contributed greatly to the fusion of electrical modalities and optical modalities, however, were not free from expensive materials such as gold (Au) and platinum (Pt) used in electrodes and conductive tracks. Another significant challenge of metal-based neural interfaces is photoelectric artifacts, which are electrical noise produced when light is applied to a metal surface. Photoelectric artifacts contaminate electrophysiological data, which explains the importance of designing transparent electrode arrays through promising candidates such as indium tin oxide (ITO), carbon-based materials or conductive polymers. ITO is a transparent conducting oxide, which has good conductivity and high transparency so that have made it broadly used in optoelectronics. Nevertheless, ITO suffers from poor mechanical properties and relatively high cost and is thus difficult to apply to flexible neural prosthetics. In addition, due to the scarcity of indium, a major component of ITO, there is a major limitation to a practical application as an implantable device from its high cost. With its high optical transmittance and reliable mechanical and chemical stability, graphene is a commonly used carbon-based material for transparent neural interfaces. However, it has limitations of the complex electrode fabrication process and relatively high electrochemical impedance. Because graphene is chemically deposited on copper foil to obtain superior crystallization, the fabrication method requires an additional “graphene transfer process” that other transparent material candidates do not. Moreover, even small defects rupture graphene layer during the transfer process, which often gives rise to a low device yield. This property hinders the potential application for developing multi-channel electrode arrays exploited by neural implants. Furthermore, the graphene-based neural interface cannot avoid trade-offs in the performances between conductivity and transparency. For example, the conductance of the electrode array can be increased by forming multi-layer graphene, thereby lowering the transparency of the electrode.

To overcome these issues, we introduce a transparent, conductive polymer-based neural electrode array with a facile fabrication method with an ultra-low cost. We chose poly(3,4-ethylenedioxythiophene) polystyrene sulfonate (PEDOT:PSS) as the electrode material for its high transparency and mechanical flexibility, maintaining its conductivity. PEDOT:PSS has been mainly deposited on film through electroplating techniques to enhance the electrochemical impedance of metal electrodes or minimize mismatches between biological tissue and electrodes. In this work, we present a low-cost, facile lift-off process by ultrasound with directly spin-coated PEDOT:PSS dispersion. In contrast with a fabrication of graphene electrodes, this approach is highly versatile as it does not require an additional transferring process. Besides, dipping the electrode array into EG changed its morphology and dramatically improved the device electrochemical impedance while maintaining its transparency (85%). EG improved the conductivity of the PEDOT:PSS electrode more efficiently than other well-known surface treatment candidates, such as nitric acid or dimethyl sulfoxide (DMSO). Finally, the high optical transparency of PEDOT:PSS made free from the severe photoelectric artifacts of the conventional Au microelectrode array, both in vitro and in vivo. As a result, the EG treated PEDOT:PSS (PEDOT:PSS-EG) electrode array will pave the way for a transparent conductive polymer-based neural interface for analyzing or treating brain neural activity.

2. Results and Discussion

2.1. Facile Fabrication of the Transparent PEDOT:PSS-EG Electrode Array

Figure 1a is a schematic diagram of the transparent PEDOT:PSS-EG electrode array. The device consists of 16 polymeric channels on the transparent polyethylene terephthalate (PET) substrate. The device is in contact with the rodent cerebral cortex, and a 473 nm blue laser, applied directly to the top of the electrode, stimulates light-sensitive, genetically modified neurons. Figure 1b is an optical image of a flexible device wrapped around a 5-mm diameter rod. The inset image shows transparency of the electrode array compared to that of an opaque Au neural electrode array. An optical microscopy image shows the desirable micrometer (∼20 μm) patterns of the interconnected lines (Figure S1, Supporting Information). Blue pristine PEDOT:PSS electrodes turn bright yellow after the EG treatment that enhances the electrode conductivity. The re-crystallization of PEDOT molecules with PSS depletion may explain this change. Regardless of this color change, the PEDOT:PSS-EG electrode array remained free from photoelectric artifacts with high transparency, as shown in Figure 1c. The fine patterning of the electrode array come from the fabrication process shown in Figure 1d. First, the photoresist is spin-coated on a PET substrate and patterned in the shape of a predesigned electrode array. The dimension of the electrode site was 300 × 300 μm², with a total thickness of the device is about 25 μm including substrate. Next, PEDOT:PSS dispersion coating is followed by the hydrophilic surface treatment with O₂ plasma. To remove the rest of the photoresist, we immersed the film in acetone and then subjected it to the lift-off process by ultrasound. After dipping the film into the EG solution about 30 min, the flexible PEDOT:PSS-EG electrode array is formed. Additionally, the device is encapsulated with UV curable epoxy (SU8 2, thickness: 2μm) except for the electrode to determine the sensing sites for

I.-J. Cho
School of Electrical and Electronics Engineering
Yonsei University
Seoul 03722, Republic of Korea
I.-J. Cho, K. J. Yu
Yonsei-KIST Convergence Research Institute
Yonsei University
Seoul 03722, Republic of Korea
electrophysiological signal recordings. The flexible array is available by simply being delaminated from the glass substrate. We also successfully fabricated a 384-channel large-area and high-density electrode array for large animal neural implants or for wearable applications (Figure S2, Supporting Information).

Figure 1e shows the transmittance versus visible wavelength plot for other representative transparent neural interface candidates: Au thin film (10 and 20 nm thickness) and bi-layered graphene. Our array exhibited higher optical transmittance (≈85%) at wavelengths from 400 to 700 nm than the bi-layered graphene electrode array (≈80%). Since the PET substrate (reference) transmittance is ≈87%, this transmittance data may be related to the opacity of the substrate itself. After the EG post treatment, negligible transmittance change was observed at the range of the visible wavelength (400–700 nm). Since 10 and 20 nm Au deposited on PET film displayed poor optical transmittance (≈30%), we also compared the optical transmittance of our device with other transparent electrode arrays for biomedical applications[8,9,13b,18,21] (Figure S3, Supporting Information). For high-quality electrophysiological signal recording, low electrochemical impedance with the consistency of implantable electrodes has significant implications.[22] We measured the electrochemical impedance according to the frequency of the 16-channel PEDOT:PSS-EG electrode array (Figure 1f). Electrochemical impedance depending on the electrode size was also measured to confirm the feasibility of further miniaturization.
of the electrode array (Figure S4, Supporting Information). We analyzed the ionic current between the electrode surface and the electrolyte by dipping the device into a phosphate-buffered saline (PBS, pH 7.4 at room temperature) solution. The impedance value of PEDOT:PSS-EG ranged from 50 to 70 kΩ at 1 kHz on average, suggesting that PEDOT:PSS-EG is suitable for reliable neural recording. To investigate whether PEDOT:PSS-EG electrode array is also compatible for electrophysiological recording of local field potential (LFP), we collected the electrochemical impedance spectra from additional 0.1 to 100 Hz (Figure S5, Supporting Information). The result showed the potential for use as a transparent implantable device at low frequencies as well as high frequency bands.

2.2. EG-Induced PEDOT:PSS Morphological Change

Figure 2a shows schematics of the EG-induced PEDOT:PSS morphological change to explain how enhances electrochemical impedance of the pristine PEDOT:PSS. Consecutive ionic bonds of conductive, hydrophobic PEDOT cores and non-conductive water-soluble PSS shells stabilize PEDOT:PSS. The pristine PEDOT:PSS film is an uneven arrangement of coiled PEDOT and PSS molecules. Since insulating PSS shells limit the pathway between the PEDOT cores where electrons can drift, the pristine PEDOT:PSS film has a relatively low electrical conductivity. Adding EG to this film switches most PEDOT chains with resonance structures from benzoid to quinoid forms. Here, the benzoid form favors the coiled conformation while the quinoid form favors the linear conformation of molecules. Thus, treating PEDOT:PSS with EG dramatically reduces the charge-trapping-associated defects and recrystallizes the cores, thereby dramatically enhancing the film's conductivity.

Figure 2b shows a scanning electron microscopy (SEM) side-view image of a PEDOT:PSS film and a PEDOT:PSS-EG film. Pristine PEDOT:PSS and EG-treated PEDOT:PSS dispersions had total thicknesses of 100 and 40.7 nm, respectively. This result suggests that the EG molecules depleted the hygroscopic PSS shells, reducing the thickness of the entire film while inhibiting severe conductive deterioration.

To investigate whether EG post-treatment enlarges the grains of the entire electrode, we produced SEM images of the surface of each film (Figure S6, Supporting Information). The images display the rearranged PEDOT-rich granules (right) after EG treatment compared with the pristine PEDOT:PSS (left) from rough black cracks.

Figure 2c shows 3D atomic force microscopy images (2 × 2 μm² scan range) of a pristine PEDOT:PSS and a PEDOT:PSS-EG film, confirming the morphological mechanism mentioned above. Notably, the EG-treated PEDOT:PSS film had bigger granulated particles than pristine PEDOT:PSS, indicating that EG treatment increased the surface roughness. Surface topological images from each film with detailed statistics also showed that PEDOT:PSS-EG, with a re-organized molecular structure, had rougher surfaces (Figures S7, S8, Supporting Information).

The electrochemical impedance of this enhanced electrode array was superior to that of bi-layered graphene electrodes with the same electrode size (300 × 300 μm²), as shown in Figure 2d. Although it displayed a slightly lower electrical conductivity than the transparent ITO electrode array, our array can be an attractive alternative given that the brittleness of ITO makes it unusable as flexible neural implants. Au-based electrode arrays showed the lowest electrochemical impedance in all ranges of frequency but had a poor optical transmittance (≈30%).

There are other material candidates to enhance the as-casted pristine PEDOT:PSS film such as nitric acid, DMSO, and methanesulfonic acid by doping or post-treatment. To confirm the optimal material that is the most compatible with the electrode fabrication process that we demonstrated, we measured the electrochemical impedance by surface post-treatment of PEDOT:PSS electrode array.

We analyzed impedance change to demonstrate that EG yields the highest conductivity (Figure 2e). Besides, as shown in Figure 2f, the device displays exceptional mechanical stability, maintaining almost constant electrochemical impedance (50 to 56 kΩ) at 1 kHz even after being wrapped and bent on a cylindrical rod (diameter: 0.5 mm) for 1000 times.

To assess the brain biocompatibility of our device, we carried out a cell proliferation experiment for 6 days on primary cortical neurons. Our device exhibited excellent biocompatibility on cortex neurons with a negligible statistical difference with glass control sample (Figure 2g; Figures S9, S10, Supporting Information). We followed a reported protocol for the primary culture of cortical neurons. Representative confocal fluorescence images from cultured neurons on both PET substrate and PEDOT:PSS-EG electrode array showed there was no statistically significant difference in neural cell viability when compared with glass substrate, even after 6 days of testing (Figure 2h,i). Further investigation of biocompatibility with PC12 cell was also conducted for the cell proliferation test referred to previous research, owing to its neuron-like property with nerve growth factor. Our device demonstrated excellent biocompatibility for 7 days, confirming that it can serve as an implantable device. The PEDOT:PSS-EG electrode array also exhibited excellent biocompatibility (87% cell viability) on NIH-373 cells in Dulbecco’s Modified Eagle Medium (Figures S11, S12, Supporting Information).

2.3. In Vitro Light-Induced Artifact Evaluation

Figure 3a shows schematic illustrations of a traditional opaque metal electrode array and a transparent PEDOT:PSS-EG electrode array with a biological interface. It highlights two main problems with the optogenetic integration of opaque electrodes: (1) photoelectric artifacts and (2) reduced light transmission efficiency. Applying a light source to a metal surface generates an electrical field that contaminates the electrophysiological signals. Moreover, metal surfaces absorb light, reducing its intensity and deteriorating the neurostimulation.

We measured the electrical signals from photoelectric artifacts on Au and PEDOT:PSS electrode arrays in PBS solution to confirm this problem. We compared photoelectric artifacts from thin, semi-transparent conventional Au electrodes (20 nm thickness) with transparent PEDOT:PSS-EG electrodes, to distinguish photoelectric voltage-induced signal more clearly.
Figure 2. EG-induced PEDOT:PSS morphological and electric characteristics changes. a) Schematic illustrations of the EG-induced PEDOT:PSS morphological change. The device dipping time was 30 min. The magnified picture illustrates how EG changes conductivity at a molecular level. b) A side-view SEM image of a 25-µm thick PET substrate coated with pristine PEDOT:PSS film (left) and PEDOT:PSS-EG film (right). The PEDOT:PSS was 100 nm thick. Scale bar: 100 nm. c) 3D AFM images of the surface of the Pristine PEDOT:PSS film (left) and PEDOT:PSS-EG film(right) on PET substrate. The scale in vertical and horizontal scan range were 8 nm and 2 µm, respectively. The arithmetic average roughness (Ra) of the pristine and EG-treated films were 1.101 and 1.484, respectively. d) Comparison plot of the electrochemical impedance of candidates for transparent electrode arrays. PEDOT:PSS (red) showed lower electrochemical impedance than that of graphene (blue) at all frequency intervals. e) Comparison plot of the electrochemical impedance of the surface treatment materials. The red line highlights our device. f) Electrochemical impedance changes after bending the microelectrode array (1000 cycles). g) Bar graph displaying the viability of the cortical neurons cultured on glass, PET, and PEDOT:PSS-EG at day 2 and 6. The single-group 3D neural network model at days in vitro (DIV) 14 for observing cell viability (ns p = 0.4214 (glass-PET, day 2), ns p = 0.8010 (PET-PEDOT:PSS-EG, day 2), ns p = 0.2387 (glass-PET, day 6), ns p = 0.1797 (PET-PEDOT:PSS-EG, day 6), n = 3, n is the number of the sample. h) Representative confocal fluorescence images of live (green) and dead (red) cells stained with calcein-AM and PI on the glass, PET, and PEDOT:PSS-EG at day 6. Scale bar: 50 µm. i) Representative confocal fluorescence images of immunostained neural bodies and neurites (Tuj-1, green) and cell nucleus (DAPI, blue) on the glass and PEDOT:PSS-EG at day 6. Scale bar: 50 µm. Data are presented as mean ± SD. Statistical significance was tested with two-tailed unpaired t-tests.
**Figure 3.** In vitro light-induced artifact evaluation. a) Schematic illustration of the conventional metal electrode array (Au) (left) and PEDOT:PSS-EG electrode array upon illumination with a blue laser pulse. Neurons appear in green. b) Experimental settings for in vitro photoelectric artifact evaluation. The 473 nm blue laser was applied directly to the target electrode with a droplet of the PBS. The optical sensor detects the transmitted light intensity through the electrode. A magnified image presents the illuminated spot during the experiment. c) Photoelectric artifacts for the Au electrode (left) and transparent PEDOT:PSS-EG electrode (right) at various light densities. d) Comparison between photoelectric artifacts for illuminated (red) and not illuminated (blue) spots. The Au electrode array (left) presented photoelectric artifacts with an amplitude of ≈200 µV, whereas the transparent PEDOT:PSS-EG electrode array (right) presented artifact-free electrical signals.
Figure 3b shows the experimental setting of the in vitro artifact evaluation process by illuminating a spot with the 473 nm blue laser pulse (fiber diameter: 105 µm, duration: 20 ms, 10 Hz). Detailed images of the experimental settings are shown in Figure S13, Supporting Information. We applied the pulse directly to the electrode array surface, then measured the light intensity in real time with an optical sensor. Next, we covered the device with a PBS solution droplet to mimic neural interface conditions. The light intensity detected by the optical sensor is proportional to the optical transparency of the electrode array. Figure 3c presents the electrical signal obtained from an electrode illuminated by the laser according to the light intensity. The transparent PEDOT:PSS-EG electrode produced an artifact-free, low-noise electrical signal even when applying a pulse with a light density of 80.88 mW mm$^{-2}$ to its surface. In contrast, illuminating the Au electrode with a light density of 28.89–80.88 mW mm$^{-2}$ produced striking artifacts.

We applied a laser of 900 mW mm$^{-2}$ to the devices to confirm this phenomenon and measured the output voltages (Figure 3d). In the non-illuminated spot, we only observed external noise within $\pm$20 µV on average for both the conventional Au electrode and the transparent PEDOT:PSS-EG electrode. However, the directly illuminated spot of the Au electrode had photoelectric artifacts of up to $\pm$200 µV. This result implies that corrupted electrophysiological data from the light-induced artifacts by opaque neural implants can significantly hinder the biological interpretation.

### 2.4. In Vivo Seizure-Like Wave Recording/2D Electrophysiological Mapping with Optogenetics

Before in vivo experiment, we first investigated the long-term stability in a biological environment of our device. The reliability of encapsulated electrode array was confirmed by electrode failure from immersing the device into the 60 °C of PBS solution (Long-term stability acceleration test). The overall lifetime of the electrode array was evaluated using the Arrhenius reaction equation, which describes the increased temperature accelerated the chemical reaction of an interface. The device did not fail for 8 days of high temperature, aqueous environment, which allowing the potential use of chronically operating implantable tools (Figure S14, Supporting Information). Further accelerated soaking test of PEDOT:PSS-EG electrode array in 60 °C artificial cerebrospinal fluid (a-CSF) was also demonstrated to address the issues of protein adsorption of an electrode interface. As a result, the device showed negligible performance degradation in a high-temperature proteinaceous solution over time, as well as physiological environment (Figure S15, Supporting Information). And then, we recorded signals from the cerebral cortex of a Thy1-ChR2-YFP mouse, inducing a seizure with pilocarpine injection to confirm the electrophysiological signal readout performance of our device (Figure 4a). Pilocarpine is associated with the M1 muscarinic receptor involved in seizure generation and activates muscarinic receptor as an acetylcholine receptor agonist. We first conformally contacted the electrode array on the right hemisphere of the mouse brain and injected the drug into the peritoneal cavity of the transgenic mouse (intraperitoneal injection). Figure S16, Supporting Information demonstrates the detailed experimental setup for electrophysiological recording. Figure 4b presents the recorded electrophysiological data from one of the channels when injecting the drug in two separate doses. The first pilocarpine injection (150 mg) induced negligible seizure-like activity. After 10 min, we injected an additional pilocarpine dose (100 mg) that noticeably induced an absence seizure-like biological signal. In the absence seizure, the spike-and-wave pattern lasts more than 2 s, mainly for generalized wave patterns of 2.5 Hz or more. Therefore, we analyzed the frequency spectrum for electrocorticography (ECoG) between 10 and 15 min after drug injection, including the seizure onset (Figure 4c). Notably, the electrophysiological signal displayed drug-evoked LFPs between 0 and 20 Hz at the time of reaction to the drug. Clear spontaneous LFPs were also measured even after the further miniaturization of the electrode sensing site (electrode size: 50 x 50 µm$^2$) (Figure S17, Supporting Information). This data suggest that our transparent PEDOT:PSS-EG electrode array can reliably capture pathological neural signals from transgenic mice.

To confirm that opaque metal electrode arrays block light in genetically modified mice, we validated the optogenetic stimulation effectiveness of the Au electrode (20 nm) and PEDOT:PSS-EG electrode arrays. The frequency of action potentials from neurons can be adjusted using the channelrhodopsin-2 (ChR2) photo-sensitive cation channel by applying a 473 nm blue laser. Figure 4d shows optical images of the Au electrode array and the PEDOT:PSS-EG electrode array implanted in the cerebral cortex of Thy1-ChR2-YFP mice. We applied a 1 mW blue laser pulse (fiber diameter: 105 µm, duration: 50 ms, 2.5 Hz) directly above the electrode sensing spot. The movie frame sequences of colormaps (Figure 4e, voltage ranges: 0–700 µV absolute values of the responses) of the devices demonstrate the difference of light transmission effectiveness between Au and PEDOT:PSS-EG. The 2D electrophysiological mapping revealed that optogenetic stimulation-induced action potentials last about 15 ms (Movies S1, S2, Supporting Information). For Au electrodes, the maximum amplitude of the modulated action potential induced by a 1 mW laser pulse was $\pm$250 µV. Because the Au surface reflected most of the light, we observed negligible potential enhancement. In contrast, irradiating a light with the same intensity onto our array yielded action potential signals of over 700 µV in the stimulating spot. This result suggests that the excellent optical transmittance of our device has resulted in successful photo-stimulation with minimal light loss.

### 2.5. In Vivo Optogenetics Depending on Light Intensities

We also conducted simultaneous electrophysiology recording and optogenetic stimulations with different laser intensities. Figure 5a,b is an anatomical schematic and an image showing where we implanted the device on the right hemisphere of a Thy1-ChR2-YFP mouse’s cortex. We changed the light pulse intensity by alternating the power of a blue laser (473 nm) targeting the electrode with a fiber (diameter: 105 µm, duration: 50 ms, 2.5 Hz) right above the channel, as presented in Figure 5c. We assessed the peak amplitude of the neural signals from the Au and PEDOT:PSS-EG arrays at each laser power.
Figure 4. In vivo seizure-like electrophysiological signal recording with 2D mapping of light-stimulated neural signals. a) Schematic illustration of the in vivo electrophysiological signal recording setup. 250 mg of pilocarpine was injected into a Thy1-ChR2-YFP mouse after device implantation. b) Electrophysiological signals from the transgenic mouse over time of pilocarpine administration. Absence seizure was evoked about 15 min after drug injection. c) Magnified timelines of the seizure-like reactions onset (top) and frequency spectrum (bottom) by highlighted time periods from (b). d) Images of device implantation for optogenetics. The blue laser was applied to the Au electrode (top) and PEDOT:PSS-EG electrode (bottom), which were in conformal contact with the cerebral cortex of a transgenic mouse. Scale bar: 600 µm. e) 2D electrophysiological mapping with 16-channel electrode array of the Au electrode (top) and PEDOT:PSS-EG electrode (bottom). Each pixel corresponds to an electrode. The total time-period for recorded and mapped data were 15 ms with an identical color map potential range (0–700 µV). All the data were color-map coded with absolute values of light stimulated evoked responses.
Figure 5. In vivo optogenetics and with different light intensities in a transgenic mouse and light-induced artifact evaluation. a) Illustration of the location of the device implanted in the right hemisphere of the cortex. The stimulated position is presented in red. b) A photograph of a device (Au electrode array) implantation in a transgenic mouse. An anisotropic conductive film connects the device to the external electrophysiological measurement system (INTAN RHD 2000). c) Side view images of blue laser ON/OFF. The waveguide fiber (diameter: 105 µm) is fixed with a stereotactic instrument. d) Measured 16-channel electrical signals from the cortex of a mouse with different light densities. Blue, green, and red lines correspond to illuminating light densities of 237.09 (2 mW), 115.55 (1 mW), and 23.11 mW mm⁻² (0.2 mW), respectively. The illuminated target electrode is depicted in light blue. In vivo light-induced artifact time-series evaluation in a wild-type mouse for the Au electrode and PEDOT:PSS-EG electrode e) without and f) with laser stimuli. Signals from the illuminated electrode are highlighted in red, and those from the not illuminated one in light blue. The light power density of the laser pulse was 120 mW mm⁻².
intensity.\cite{footnote1} Figure 5d shows representative peak values of 16-channel Au electrodes and PEDOT:PSS-EG electrodes with a light density of 23.11, 115.55, and 237.09 mW mm$^{-2}$. Figure S18, Supporting Information provides a detailed specification of the laser pulse intensity. Stimulating the site of the Au electrode array with a 23.11 mW mm$^{-2}$ light did not cause ChR2-triggered neuromodulation. Moreover, the Au electrode surface absorbed most of the light in the other light density stimuli, and we measured a peak value of $\approx 200 \mu$V. In contrast, the transparent PEDOT:PSS-EG electrodes showed exceptional neuro-stimulus values even at 23.11 mW mm$^{-2}$.

To assess the sensitivity of our array, we applied lights of density 11.55, 5.78, and 3.47 mW mm$^{-2}$ to the target electrode. A 5.78 mW mm$^{-2}$ density was sufficient for the highly transparent PEDOT:PSS-EG to induce light-stimulated potentials on a transgenic mouse (Figures S19, S20, Supporting Information). We also compared the photoelectric artifacts between the Au electrode and our transparent electrode upon light stimulation in wild-type mice. The ECoG signals for the 16 channels of the Au electrode array allowed us to distinguish between neural signals and photoelectrical artifacts (Figure S21, Supporting Information). Notably, we observed electrical noise of about 170 $\mu$V of amplitude with the Au electrode, whereas our transparent electrode array recorded only ECoG signals from the mouse (Figure 5e,f). We also confirmed that the conventional metal electrode produced topical photoelectric artifacts by discriminating the illumination spots of both electrode arrays. These results confirm that our transparent electrode is more sensitive and less subject to artifacts than conventional metal electrodes. This result confirms our device potential as a candidate for robust integration of electrophysiology with optogenetics.

3. Conclusion

This study introduced a PEDOT:PSS-EG transparent electrode array for simultaneous neural recording and optogenetics. Low cost and facile fabrication of such electrode array suggests the capability of producing large area neural devices and mass production of the electrodes, thereby enabling the high-fidelity neural recordings with optical modalities in a most convenient way. We demonstrated that the EG dipping process induced a morphological change that dramatically reduced the electrochemical impedance of the pristine PEDOT:PSS electrode array. Compared with the thin film of the semi-transparent Au electrode, the device presented here exhibited excellent optical transparency in both in vitro and in vivo environments, allowing us to record photoelectric artifact-free signals. Our electrode array also showed prolonged operation time in proteinaceous environment, which demonstrates capabilities of being applied for chronic implantable tool, not only the acute electrophysiological recording. However, further extension of the device failure time should be developed, changing the intrinsic characteristics of PEDOT:PSS or encapsulation for follow up research. Our electrode array has high transparency but has a relatively higher electrochemical impedance than the existing metal-based electrode, so if the size of the electrode becomes smaller, it would be difficult to measure the single spike activity. So further investigation to enhance the electrical properties of electrodes maintaining their optical transparency is needed. Furthermore, our device is fully transparent which consists of transparent electrical traces with PEDOT:PSS-EG interconnects. This configuration may give rise to a limitation of the minimum electrochemical impedance. However, this study presents a promising transparent electrode array design could lead to further integrations with multiple modalities such as electrical stimulation with neural recording, thus expanding neuroscience research possibilities.

4. Experimental Section

Materials and Equipment: 23-µm PET transparent film (ES301230) was purchased from CFM Korea. 1.3 wt% conductive PEDOT:PSS dispersion in H$_2$O was purchased from Sigma Aldrich, and 99% EG from Hayashi. All materials were used as received. The transmittance of the transparent electrode array and other control samples was obtained using a spectrophotometer (FC-PH10 Cary 5000). Electrochemical impedance of devices was characterized using a Gamry Reference 600+ potentiostat (Gamry instrument). SEM (FL-CM20 CD-SEM II) was conducted to scrutinize the surface and side-view of Pristine PEDOT:PSS and EG-treated PEDOT:PSS. AFM (NX-10, Park Systems) image was obtained from Yonsei Center Research Facilities. Electrical signals from the device in PBS solutions and the electrophysiological signals from the implanted device were acquired on an Intan (RHS 2000, Intan technologies). A laser instrument (ADR-2301, RGBLase LLC, California, USA) for 473 nm blue laser stimulation in vitro and in vivo was used.

Fabrication of the PEDOT:PSS-EG Electrode Array: To make the temporary substrate, glass slides were spin-coated with polydimethylsiloxane (PDMS) (Dow Corning) at 2000 rpm. The glass was next laminated with the flexible PET substrate. A positive photoresist (AZ 5214-E, AZ Electronic Materials, USA) was then used for photolithographic patterning of the device. AZ 5214-E was spin coated on the PET substrate (rotation speed: 3000 rpm, soft bake: 2 min at 110 °C, UV expose time: 10 s). The device was then patterned by immersing it into developer solution for a 1 min (AZ 300 K, AZ Electronic Materials, USA). Because PEDOT:PSS is hydrophilic, the surface of the device was first treated at 100 W, 40 SCCM of O$_2$ for 25 s for spin-coating of PEDOT:PSS solution. After spin-coating the PEDOT:PSS solutions at 1000 rpm, the film was dried at 110 °C on a hot plate, followed by ultrasonication process for a lift-off resist, except for the PEDOT:PSS electrode array. Before electrode encapsulation, the surface of the film was treated with O$_2$ plasma (100 W, 50 SCCM, 3 min). For encapsulation, the negative photoresist (SU8-2, Kayaku Advanced Materials) was spin coated on the oxygen treated surface (rotation speed: 2000 rpm, soft bake: 1 min at 65 °C and 1 min at 95 °C, UV expose time: 8 s, post-exposure bake: 1 min at 65 °C and 1 min at 95 °C). The encapsulated film was then immersed in the SU-8 developer (SU8 developer, Kayaku Advanced Materials) to determine the electrode contact via. The device was completed by slightly delaminating the film from the glass substrate. For In vitro and In vivo experiment, the border of the devices was cut with a femtosecond laser (s-pulse HP, Amplitude, France) to implant them in the mice cortex (2.4 mm x 3 mm).

Fabrication of the Conventional Metal (Au) Electrode Array: First, the PDMS (Dow Corning) was spin-coated at 2000 rpm on the temporary slide-glass substrate. Then the PET flexible substrate was laminated on the slide glass. Next, 3 nm thickness of the chromium (Cr) was deposited on the PET substrate for adhesion layer with thermal evaporator (KVE-T2000, Korea Vacuum Tech.). And then the 20 nm thickness of the Au was deposited on the adhesion layer. Next, spin-coating of the photoresist (AZ 5214-E, AZ Electronic Materials, USA) was followed (rotation speed: 3000 rpm, soft bake: 2 min at 110 °C, UV expose time: 10 s). The photoresist was then patterned by dipping in the developer solution for a 1 min (AZ 300 K, AZ Electronic Materials, USA). Next, the device was immersed in an Au etchant (CHM00D679, Transene Company, Inc.) and a Cr etchant (CHM00D673, Transene Company, Inc.) to form individual electrode array.
and conductive tracks. After the treatment of the surface of the device at 100 W, 40 SCCM of O₂ for 25 s, the UV-curable negative photore sist (SU-8 2, Kayaku Advanced Materials) was spin-coated for encapsulation of the device (rotation speed: 2000 rpm, soft bake: 1 min at 65 °C and 1 min at 95 °C, UV expose time: 8 s, post-exposure bake: 1 min at 65 °C and 1 min at 95 °C). The encapsulated film was then immersed in the SU-8 developer (SU8 developer, Kayaku Advanced Materials) to form electrode via for contact with brain tissue. After the hard bake of the SU-8 2 (110 °C at 1 h), the completed device was slightly delaminated from the slide-glass substrate.

**Electrochemical Impedance Spectroscopy:** Electrochemical impedance spectroscopy (EIS) was measured on a Gamry Reference 600+ potentiostat (Gamry instrument). Impedance with an Ag/AgCl reference electrode and a large area platinum electrode for counter electrode with the working electrode for experimental devices was obtained (three-electrode configuration). The sweep frequency was 100 Hz–100 kHz with an alternating current measuring voltage of 10 mV, for devices immersed into PBS (pH 7) solution. The oscillating amplitude was fixed at 10 mV and points per decade were 10.

**Bending Test of the Device:** Bending test was performed by the device wrapped around a cylindrical rod which was a diameter of 0.5 mm. After each bending cycles, electrochemical impedance of the device was measured on a Gamry Reference 600+ potentiostat (Gamry instrument). Same experimental protocols were adopted that of EIS (sweep frequency: 100 Hz–100 kHz with an alternating current measuring voltage of 10 mV).

**Primary Neural Culture:** Pregnant Sprague Dawley rats (embryo 18) were purchased from DBL Co. (South Korea) and sacrificed for 2D neural cultures on the glass substrate, and PET film with PEDOT:PSS-EG pattern. The cerebral cortex was dissected from the brain of decapitated embryos. Then, the extracted tissue was treated and triturated with the enzyme mixture from neural tissue dissociation kits (Miltenyi Biotec, Germany) using the gentleMACS dissociator (Miltenyi Biotec, Germany). The isolated live neurons were manually counted through a Trypan blue (GIBCO, USA) using a hemocytometer (INCYTO, South Korea). Then, the neurons were plated at a density of 400 cells mm⁻² on the two substrates (i.e., glass substrate and PET film with PEDOT:PSS-EG pattern) coated with 100 µg ml⁻¹ of poly-D-lysine (Sigma Aldrich, USA). The neurons were cultured in the culture medium, consisting of neurobasal Plus medium supplemented with 2% v/v B27 Plus supplement (Invitrogen, USA), 2 mM Glutamax-I (GIBCO, USA), and 1% v/v penicillin-streptomycin (P/S; GIBCO, USA) and maintained in a CO₂ incubator at 37 °C.

**Live/Dead Cell Viability Assay of the Cortical Neurons:** The viability of the cortical neurons was assessed at 2 and 6 days in vitro (DIV). The samples were treated in 1x PBS, containing 1 µM CellTrace calcein green, AM (calcein-AM; Thermo Fisher Scientific) and 15 µM propidium iodide (PI, Sigma Aldrich, USA), at 37 °C for 30 min. After washing with 1x PBS, fluorescence micrographs that displayed live (green) and dead (red) cells were acquired using a confocal laser scanning microscope (LSM 700, Carl Zeiss, Germany).

**Immunostaining and Imaging of Cortical Neurons:** To visualize the morphologies (i.e., neuronal cell bodies and neurites) of the cortical neurons cultured on the glass and PET film with PEDOT:PSS-EG, cortical neurons were stained with neuron-specific class III beta-tubulin (mouse anti-Tuj-1, 1:1000, T8678, Sigma Aldrich, USA) antibody. First, the samples were fixed in 4% v/v paraformaldehyde in 1x PBS for 1 h at room temperature. After washing with 1x PBS three times, each for 30 min, the samples were blocked in a blocking solution, consisting of 0.1% v/v Triton X-100 and 3% v/v bovine serum albumin in 1x PBS for 2h at room temperature. After washing with 1x PBS three times, each for 30 min, the samples were treated with the Tuj-1 antibody in the blocking solution for 2h at 4 °C. After washing with 1x PBS three times, each for 30 min, the samples were treated with secondary antibody (goat anti-mouse conjugated Alexa Fluor 488, 1:1000, A-11001, Invitrogen, USA) in the blocking solution for 2h at room temperature. After washing with 1x PBS three times, each for 30 min, the samples were also treated with 4′,6-diamidino-2-phenylindole (1:1000, D1306, Invitrogen, USA) in 1x PBS for 1 h at room temperature. Finally, after washing with 1x PBS three times, each for 30 min, fluorescence images were acquired using the confocal laser scanning microscope (LSM 700, Carl Zeiss, Germany).

**Live/Dead Cell Viability Assay:** In vitro cytotoxicity assay was first demonstrated with WST-8 assay observing 450 nm absorbance. And then, cell viability was assessed using a Live/Dead Viability/Cytotoxicity Kit (Thermo Fisher Scientific, Waltham, MA, USA). PC-12 cells (Manassas, VA, USA) in Roswell Park Memorial Institute 1640 medium (Gibco, Waltham, MA, USA) supplemented with 5% fetal bovine serum (Gibco), 10% v/v horse serum (Gibco), and penicillin-streptomycin (Gibco) was cultured. Cells were incubated in 5% CO₂ at 37 °C, refreshing the media every 2 days. The cells were seeded on a glass substrate (negative) and cell culture plate (positive) at the same concentration for the controls. To grow stained cells, the culture medium was removed by suction and washed the cells with sterile PBS three times. And then, the cells were stained with 10 µL of ethidium homodimer I (Ethr-1) of 2 mm stock solution and 2.5 µL of calcine-AM of 4 mm stock solution in 5 mL of PBS solution. After incubation at 37 °C for 20 min, the stained cells were observed with a laser scanning confocal microscope (LSM 700; Carl Zeiss, Oberkochen, Germany). The live and dead cells appeared in green and red, respectively.

**In Vitro Light-Induced Artifact Evaluation:** All the experiments were conducted in a dark room. The electrode and Au electrode surfaces were induced with 473 nm wavelength light. And then, the blue laser was applied with each pulse density (5.78, 11.55, 17.33, 21.31, 28.89, 34.66, 46.22, 57.77, 69.33, and 80.88 mW mm⁻²) directly on the surface of the PEDOT:PSS-EG electrode array and Au electrode array, respectively. The light intensity from the tip of the optical fiber for stimulation on an optical power meter (1936-R, Newport Inc., Irvine, CA, USA) was also measured with a 540 photodetector (91BD, Newport Inc., Irvine, CA, USA). An implantable fiber with optic cannula of Ø105 µm Core with 0.22 NA (Thorlabs, New Jersey, USA) delivered the light. The electrical signals from the device were recorded on an Intan recording system (Intan RHD 2132 system, Intan technologies, Los Angeles, California).

**Accelerated Long-Term Device Stability Test:** The device was immersed in the 60 °C PBS and a-CSF solution after the interconnects were fully encapsulated by PDMS film, to prevent the external cable from the immerssed solution. The device EIS was measured on a Gamry Reference 600+ potentiostat (Gamry instrument). Impedance with an Ag/AgCl reference electrode and a large area surface platinum electrode for counter electrode with the working electrode for experimental devices was obtained (three-electrode configuration).

**Animal Surgery:** The Korea Institute of Science and Technology (KIST) in Seoul, Korea, approved all the procedures involving animals. All procedures were conducted following the ethical standards stated in the Animal Care and Use Guidelines of KIST. The transparent ECoG electrode array was tested on adult male transgenic mice (C57BL6 Thy1-Chr2-YFP; 8–10 weeks, 25–30 g) and adult male wild-type mice (C57BL6; 8–10 weeks, 25–30 g). The mice were anesthetized with urethane (1.5 g kg⁻¹, intraperitoneal injection) and placed on a stereotaxic device (David Kopf Instruments, USA). The skull was drilled and opened to expose the right cortex for positioning the electrode array. And then, reference wire electrode was implanted in the left hemisphere.

In Vivo Pilocarpine Electrophysiological Signal Response: Electrophysiological signals from the drug-injected experimental mice were recorded on an Intan recording system (Intan RHD 2132 system, Intan technologies, Los Angeles, California). Pilocarpine (250 mg kg⁻¹, P6503, Sigma, USA) was used to induce absence seizures. Sequential projection of 150 mg kg⁻¹, then 100 mg kg⁻¹ of pilocarpine was injected into mice with 10 min loading time. The raw signals from absence seizure were filtered and digitized through the Intan software (20 KS s⁻¹ channel, 0.1–6 kHz band-pass filter, 60 Hz Notch filter).

**Electrophysiological Recording with Optogenetics:** All the experiments were conducted in a dark room. The PEDOT:PSS-EG electrode and Au electrode surfaces were stimulated with 473 nm wavelength light. Each light pulse density was 231.09, 115.55, and 23.11 mW mm⁻². The square wave frequency was 0.5–2.5 Hz with a 12.5% duty cycle. The light
intensity from the tip of the optical fiber for stimulation on an optical power meter (1936-R, Newport Inc., Irvine, CA, USA) was measured with a 540 photodetector (918D, Newport Inc., Irvine, CA, USA). An implantable fiber with a 105-µm diameter optic cannulae Core with 0.22 NA (Thorlabs, Newton, New Jersey, USA) delivered the light. ECoG signals were recorded using the Intan recording system (Intan RHD 2132 system, Intan technologies, Los Angeles, California) and filtered and digitized the raw signal through the Intan software (20 kS·s⁻¹ per channel, 0.1–6 kHz band-pass filter, 60 Hz Notch filter).

Data Analysis of In Vitro Light-Induced Artifact: The electrical signals received by electrode array in vitro were saved as RHD files (Intan RHD 2132 system, Intan technologies, Los Angeles, California). Then data were imported into MATLAB, which could load signals in the form of “.mat”.

Electrical signals upon illumination with 5.78–80.88 mW mm⁻² blue laser for artifact evaluation was measured. The artifacts with 900 mW mm⁻² light density were analyzed depending on the illuminating spot. The timeline was 0.3 s, and the allowed voltage amplitude was −250 to +250 µV.

Data Analysis of 2D Mapping Electrophysiological Signals with Optogenetics: The colormap of electrophysiological signals was plotted by importing data with a customized MATLAB script. To associate each pixel with their respective electrodes, the data were divided into 16 channels and obtained their data in timelines. The colormap voltages were ranged from 0 to 700 µV. The total colormap visualization time was 3 s with the onset of optogenetic stimulation responses.

Statistical Analysis: Statistical analysis was performed with Origin Lab. For electrochemical impedance plot depending on the electrode size was presented as mean values ± s.d., n = 16 electrodes. For the cortical cell viability test, statistical significance was tested with two-tailed unpaired t-tests.

Supporting Information
Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements
This work acknowledges the support received from National Research Foundation of Korea (Grant no: NRF-2019R1A2C2086085, NRF-2021R1A4A1031437, NRF-2018M3A7B4047109) and Brain Convergence Research Program of the National Research Foundation (NRF) funded by the Korean government (MSIT) (Grant no: NRF-2019M3ED2A10163814). The Acknowledgements and author’s name (H.J.J.) was corrected on March 2, 2022 after initial online publication.

Conflict of Interest
The authors declare no conflict of interest.

Author Contributions
Y.U.C., J.Y.L., and U.-J.J. contributed equally to this work. Designing research: Y.U.C., J.Y.L., I.-J.C., and K.J.Y. Device fabrication: Y.U.C., J.Y.L., K.Y.K., H.J.J., J.H.P., J.W.J., and H.W.K. Data analysis: Y.U.C. and J.Y.L. In vitro experiments: Y.U.C. and U.-J.J. In vivo experiments: Y.U.C. and U.-J.J. Cell viability, biocompatibility test: S.L.L., Y.M.J., and H.G.S. Lithography optimization: Y.U.C., S.H.P., and J.Y.L. Writing—original draft: Y.U.C. Writing—review and editing: Y.U.C., I.-J.C., and K.J.Y. All authors discussed the results and commented on the manuscript.

Data Availability Statement
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords
facile fabrication, photoelectric artifact-free implants, transparent neural electrode arrays

Received: June 9, 2021
Revised: November 9, 2021
Published online: November 28, 2021
