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Polydopamine

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Electrochemically Driven, Electrode-Addressable Formation of Functionalized Polydopamine Films for Neural Interfaces**

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An array of chemically functionalized electrodes is a versatile platform for investigation/manipulation of the electroactive cellular functions that are realized in the network fashion, as well as for multiplexed detection of analytes. For example, multielectrodes have been used intensively for both perturbation of many cellular systems with electrical stimulations and measurements of electrical signals from them at the spatiotemporal resolution in the applications, such as chip-based sensors[1] and prosthetic devices,[2] along with fundamental studies of electrophysiology.[3] In particular, nerve cells are a representative example for electrically excitable cells, the functions of which are achieved by the tightly polarized electrical communications between neurons, and multielectrodes have been the core part of neuron–material interfaces.[2,4]

In vitro neuron–multielectrode interfaces have generally been formed by coating all of the electrode surfaces with one kind of neuron-adhesive molecules, such as poly(amino acid)s or peptides.[5] Although this coating approach has been successful in collective studies of neuronal connectivity and activities,[6] it would be desirable for sophisticated control and manipulation of neurons to develop methods for coating and functionalizing each electrode independently. The electrode addressability would allow us to locate or manipulate neurons or synapses with directionality control, because the electrodes are asymmetrically functionalized, not to mention being beneficial in multiplexed detection of targets. The differently functionalized electrodes on a substrate also could be utilized to investigate the responses of neurons at the single-cell level at a time.

Electrochemically driven reactions on self-assembled monolayers have been used for electrode-selective functionalizations,[7] but this approach requires a specific electrode material, depending on the self-assembling molecules, and cumbersome synthetic procedures. As an alternative, conducting polymers have been adopted for coating of neural electrodes.[8] Although they can be electrochemically deposited onto the electrodes with relative ease, while retaining electrical properties of the electrodes for neural interfaces in the reliable manner, direct functionalization is not possible owing to the lack of functionalizable groups in their structures.[9] In this work, we report the electrochemically driven formation of polydopamine (pDA) films that are substrate-independent, simple to functionalize, and site-addressable.

We have previously reported that the substrate-independent, bulk-coated pDA film did not impede the functions of the electrodes, suggesting one of the optimal materials for neural interfaces.[10] The facile functionalization of pDA films by co-deposition was also demonstrated in the bulk coating.[11] Because the polymerization of dopamine is thought to occur through oxidative coupling of 1,2-dihydroxybenzene (catechol), we reasoned that the electrochemical method[12] could be utilized to deposit pDA films selectively onto the voltage-applied electrodes (Figure 1 a). The electrochemical deposition was set to be performed at pH 6.0, because the solution-based polymerization of dopamine occurred under basic conditions (pH 8.0),[13] and this reaction should be prevented for the site-selective deposition. Prior to electrochemical deposition, we first investigated the oxidation/reduction characteristics of dopamine by cyclic voltammetry (-0.8 V to $+0.8$ V). As the main oxidation peak of dopamine was around $+0.4$ V, we used $+0.5$ V to electrochemically synthesize pDA (e-pDA) on a flat gold surface (see the Supporting Information, Figure S1, for the cyclic voltammetry of dopamine and the current measurement during the electrochemical reaction). The reaction characteristics were investigated for 1 h (Figure 1 b). The e-pDA thickness constantly increased with reaction time, while the cumulative amount of transferred charge started to reach saturation at about 25 min. This result implies that the electrochemical oxidation of dopamine occurred mainly in the first 25 min, and the polymerization of the oxidized dopamine proceeded with a relatively slower rate. In other words, the rate for the polymerization of oxidized dopamine was slower than that for its generation.

The possibility of co-deposition in the e-pDA formation (that is, functionalization of e-pDA films) was also tested by using a flat gold substrate. Poly-D-lysine (PDL) or cyclo-(RGDfC) (R arginine, G glycine, D aspartic acid, f D-phenylalanine, C cysteine) was co-deposited with dopamine (1:10 (w/w)), where the amine (NH_2) in PDL or the thiol (SH) in

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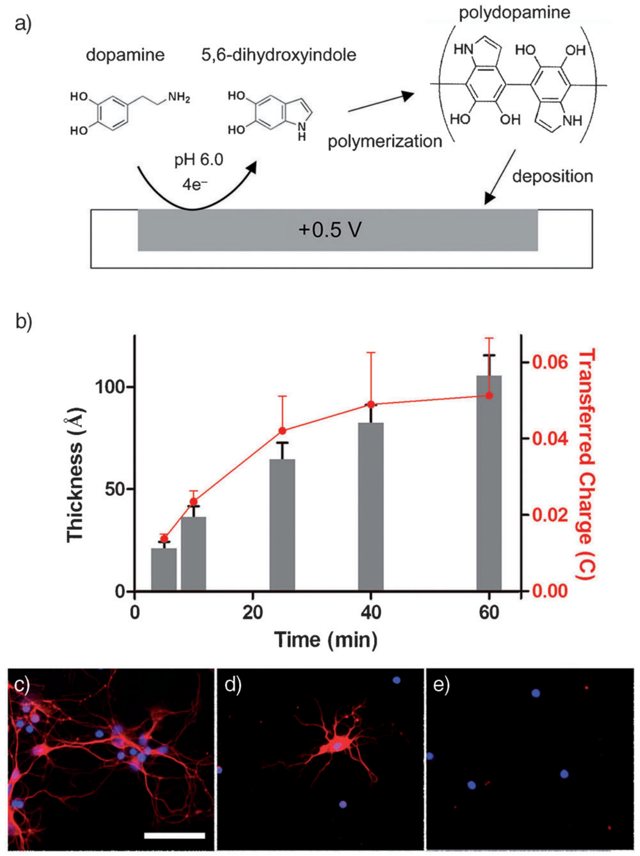

Figure 1. a) Illustration of the electrochemical deposition of polydopamine films. b) The relationship between the transferred charge and the thickness of the deposited polymer film on flat gold surfaces. The amount of the transferred charges after 5 min, 10 min, 25 min, 40 min, or 1 h reaction was 0.014 ± 0.001, 0.024 ± 0.003, 0.042 ± 0.009, 0.049 ± 0.014, or 0.051 ± 0.015 C (mean ± S.D., $n=5$), respectively. The thickness of the e-pDA film deposited during the reaction was 24.43 ± 0.95, 41.80 ± 1.01, or 114.87 ± 8.66 Å (mean ± S.E., $n=5$), respectively. c)–e) Fluorescence micrographs of hippocampal neurons on c) Poly-D-lysine (PDL)-functionalized e-pDA, d) cyclo(RGDfC)-functionalized e-pDA, and e) e-pDA. For co-deposition, a 10:1 (w/w) mixture of dopamine and PDL (or cyclo(RGDfC)) in a phosphate-buffered aqueous solution (PBS) (pH 6.0) was used. Cells were immunostained at 5 days in vitro (DIV) with anti-beta III tubulin (red) and Hoechst 33342 (blue). Scale bar: 50 μm.

cyclo(RGDfC) was covalently bonded to quinone, reportedly by 1,4-addition, in the e-pDA structures.[10,11,14] The incorporation of PDL into the e-pDA film was confirmed by X-ray photoelectron spectroscopy: the N/C ratio increased from 0.095 to 0.167 when the 1:10 mixture of PDL and dopamine was used (for details and the chemical properties of PDL-functionalized e-pDA, see the Supporting Information, Figure S2). After formation of functionalized e-pDA films, we cultured hippocampal neurons on the PDL- and cyclo(RGDfC)-functionalized films. The well-spread somata and the elongated and branched neurites clearly showed the good neuronal attachment and development on the functionalized e-pDA films (Figure 1 c and d). In contrast, the 1 h-deposited e-pDA did not support the neuronal attachment (Figure 1 e). These results indicate that PDL and cyclo(RGDfC) was linked to the e-pDA film by simple co-deposition, and this approach could be expanded easily to other molecules of interest bearing amine or thiol for functionalization of the electrodes. It also would be possible to generate an array of multiply functionalized electrodes by sequential electrochemically driven co-deposition of different molecules, where the ratio of dopamine and molecule to be co-deposited determines the surface density of the molecules.

The functionalized e-pDA films successfully acted as a neural interface. The PDL-functionalized e-pDA film was formed on a Pt-black-coated microelectrode array (MEA). The deposition was performed using a 10:1 mixed phosphate-buffered aqueous solution (PBS) of dopamine and PDL (pH 6.0) for 40 min. Neurons were plated at the density of 1000 cells mm^{-2}, and they formed a dense network on the MEA (Figure 2). For the stimulation of the cultured neuronal network, positive-first biphasic voltage pulses (duration of 200 μs) were delivered with the amplitude of +500 mV. The spontaneous activities of the neuronal network were observ-

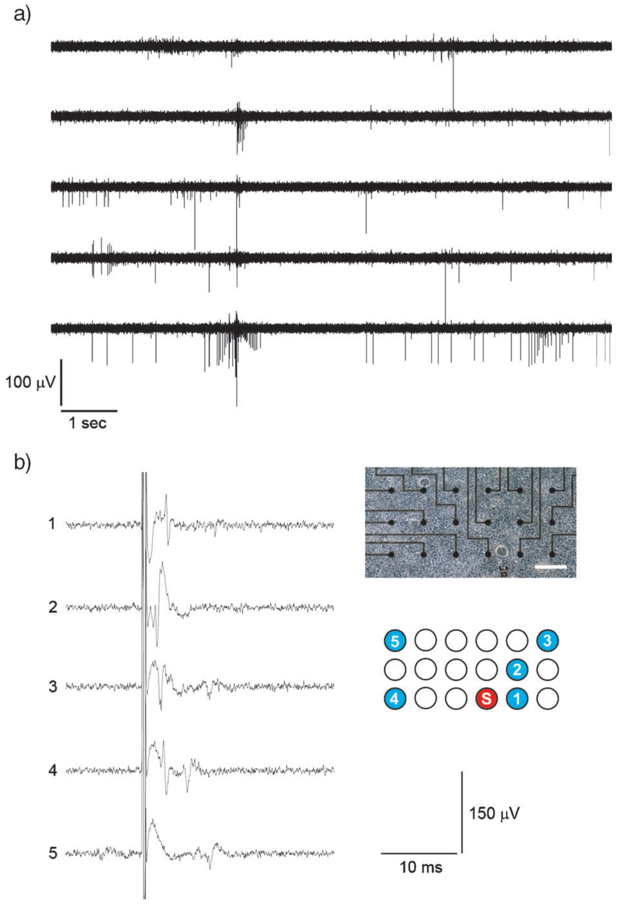

Figure 2. PDL-functionalized e-pDA films on electrodes as a neural interface. Hippocampal dissociated neural culture (12 DIV) on MEA was used for recording and electric stimulation. a) Spontaneous electric activity during 10 s; channel order was the same as that shown in (b). b) Electric stimulation-evoked neuronal responses. A positive first-biphasic-pulse (500 mV, 200 μs) was injected to the "S" electrode, and the responses recorded at the "1 to 5" electrodes were plotted on the left side. The actual phase contrast image of the neural culture is also shown as an inset (scale bar: 200 μm).

able at 15 DIV (days in vitro), as represented in Figure 2a. The mean amplitudes of spiking signals were 58, 63, 49, 62, and 75 μV_{pp}, with background noise levels of 3.67, 3.55, 3.49, 3.59, and 3.43 μV_{rms} for each trace, which resulted in the signal-to-noise ratio of 14–22. Figure 2b shows the five single traces measured by the five electrodes previously used for the spontaneous recording. The evoked responses were detected within tens of milliseconds, and the time delays of these direct responses from the stimulation slightly increased as the distance between the stimulation electrode and recording site increased. However, the responses indeed occurred even at the fairly distanced electrodes, indicating the neuronal network was successfully formed on the entire chip. These results indicated the functionalized e-pDA film did not interrupt MEA recordings or stimulations.

With all of the required properties of e-pDA films for neural interfaces in hand, the electrode-selective deposition of e-pDA films was investigated with a TiN MEA (electrode diameter: 30 μm). This MEA exhibited the low impedance value (ca. 100 kΩ), making the electrodes highly sensitive to organic adlayers, and therefore the electrode addressability could be confirmed with relative ease. The deposition kinetics was investigated by selecting two target electrodes out of 59 electrodes to be sparsely located. Voltage was applied only to the target electrodes while the voltage of the other electrodes being held to 0 V, and impedance measurement was performed every 2 min at target electrodes. The voltage of +0.5 V being applied, the target electrodes showed a gradual impedance (|Z|) increase, indicating the deposition of e-pDA films (Figure 3a, left).

The deposition was also confirmed by the gradual decrease of the impedance phase of the target electrodes (that is, becoming more capacitive; Figure 3a, right). For the further confirmation of the electrode-selective deposition, the impedance of all the 59 electrodes, including two target electrodes, was measured at 40 min after deposition of e-pDA film at +0.5 V, by which we obtained the spatial distribution of the impedance increases at each electrode (Figure 3b). The impedance magnitudes of the two target electrodes reached a 3.0- and 2.6-fold increase from the initial values, respectively, which were clearly differentiated from the behaviors of the control electrodes (1.0-to-1.3-fold increase; Figure 3c, left). The target-specific changes were also observed for impedance phases (from −55.2° to −82.7° for targets and from −58.2° to −56.2° for controls), indicating that only the target electrodes were covered by capacitive adlayers (Figure 3c, right). The electrode-specific increase of impedance magnitude constantly occurred through 40 min, which was consistent with the previously observed thickness increase on the gold substrates. All of the results clearly confirmed that the electrodes were coated with polydopamine and functionalized with molecules of interest simultaneously. This work is the first example of electrochemical formation of functionalized polymer films in an electrode-selective fashion.

In summary, we have developed a simple but versatile method for simultaneously coating and functionalizing a specific electrode in the MEA with a spatial resolution based on electrochemical polymerization of dopamine under slightly acidic conditions. The electrode-selective functionalization has a great impact to the multiplexed sensing systems and the asymmetrical manipulation of cells, which is particularly important in the neural interfaces. The functionalized polydopamine layer on the electrode was found to be suitable for neural attachment and development without a loss of the electrical properties of the electrode. We believe that the method demonstrated herein would provide a solid platform for multiplexed detection of neuronal responses as well as molecular targets.

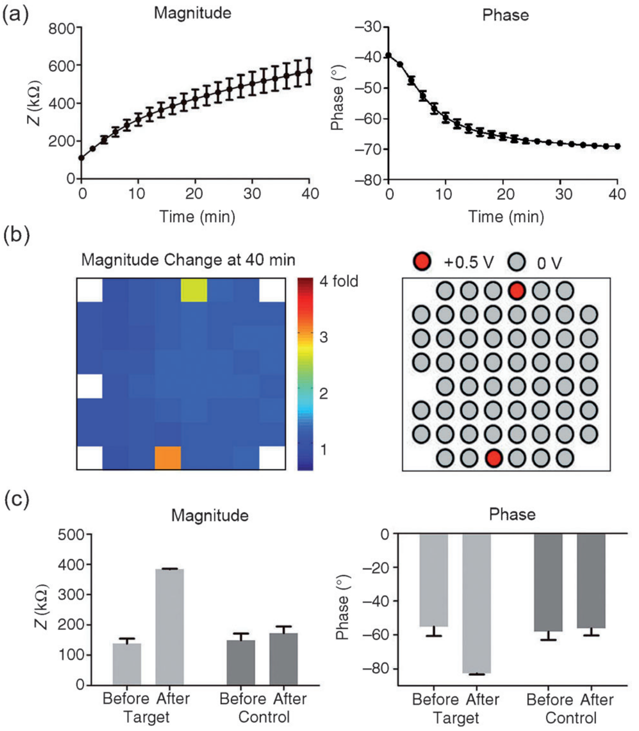

Figure 3. Impedance measurements during the electrode-selective formation of PDL-functionalized e-pDA films. a) Positive (+0.5) voltage was applied to the selected electrodes with impedance measurements every 2 min (mean ± S.D., *n* = 3). b) Color-coded spatial map of the impedance change in 59 microelectrodes (left). The color bar represents the ratio of the impedances before and after the reaction. Five white spots in the map are empty spots; electrode layout and the target electrodes are shown in right. c) Change of impedance values (magnitude and phase, mean ± S.D., *n* = 56 and 2 for targets and controls, respectively) of target and control electrodes after 40 min reaction on the TiN MEA (electrode diameter: 30 μm).

Experimental Section

Cell Cultures: Primary hippocampal neurons were cultured in serum-free condition. Hippocampus from E-18 Sprague-Dawley rat was triturated in 1 mL of Hank's Balanced Salt Solution (HBSS) using a fire-polished Pasteur pipette. The cell suspension was centrifuged for 2 min at 1000 rpm, and cell pellet was extracted. The cell pellet was suspended again in Neurobasal media supplemented with B-27, 2 mM L-glutamine, 12.5 μM L-glutamic acid, and penicillin–streptomycin using Pasteur pipette. Dissociated cells were seeded at the density of 50 cells/mm^2 on the prepared substrates. Cultures were maintained in an incubator (5% CO_2 and 37°C), and the half of media was replaced with fresh culture media without L-glutamic acid supplement every 3–4 days. This study was approved by the Institutional Animal Care and Use Committee (IACUC) of KAIST.

Instruments and Characterization: Fluorescence micrographs of neuron cultures were obtained using Olympus BX51M (Olympus Corp., Japan) equipped with a CCD camera (DP71, Olympus). From the images, the lengths of major neurites were measured with Neuron J plugin in Image J software (NIH). The morphologies of the fixed neurons were investigated by field-emission scanning electron microscopy (FE-SEM). Before the FE-SEM imaging, the samples were coated with platinum (30 mA, 360 s). TiN-MEA was purchased from Multi Channel Systems (Reutlingen, Germany).

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