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Electrodeposition of anchored polypyrrole film on microelectrodes and stimulation of cultured cardiac myocytes

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Abstract

The electrically conducting polymer polypyrrole (PPy) was electrochemically deposited onto Pt microelectrodes on a polyimide (PI) substrate. Pre-modification of the PI surface with a self-assembled monolayer of octadecyltrichlorosilane-induced anisotropic lateral growth of PPy along the PI surface and enhanced adhesive strength of the PPy film. The lateral growth of PPy film around the electrode anchored the whole film to the substrate. External stimulation of cultured cardiac myocytes was carried out using the PPy-coated microelectrode. The myocytes on the microelectrode substrate were electrically conjugated to form a sheet, and showed synchronized beating upon stimulation. The threshold charge for effective stimulation of a 0.8 cm^2 sheet of myocytes was around 0.2 μC, roughly corresponding to a membrane depolarization of 250 mV.

Keywords: Cardiomyocyte; Cell culture; Electrical stimulation; Electroactive polymer; Electrochemistry

1. Introduction

Engineering the interfacial contact between electrodes and biological cells is of central importance to the advancement of cell-based bioelectronics [1–6] and the development of neural prosthetic devices [7–11]. Extracellular electrical stimulation is still often technically challenging, especially when using smaller-sized microelectrodes, due to limitations on the current value (charge value), which can be applied without causing a faradic reaction and gas evolution [12]. Although porous platinum black has been used as a high capacity electrode [13], its brittleness is a potential serious drawback in applications for design of flexible neural probes [11]. In addition, effective interaction with cells and tissues cannot be assumed using such metal electrodes.

A conducting polymer such as polypyrrole (PPy) has a large surface area owing to its fibrous structure and thus is a high-capacity electrode material. Furthermore, the ease of preparation, inherent electrical conductivity, controllability of surface biochemical properties, and biocompatibility make PPy a promising interfacial material for use in neural prosthetic devices. Biocompatibility [7,8] and impedance characteristics [9] of PPy have been studied aiming the use for implanted medical biodevices.

In addition to in vivo applications, in vitro cellular engineering also requires suitable bio interfacing. Recent technical progress in cellular micropatterning [1–5,14–17] enables the bioassay of cellular networks combined with electrode arrays. For example, we have succeeded in preparing micropatterned neuronal PC12 cells on a microelectrode array substrate in a manner allowing alignment of the micropatterns of cells to that of electrodes [15]. Smart biointerfacing with conducting polymers will contribute to realization of these next-generation bioassay chips.

In this paper, we will firstly present the method for formation of PPy films incorporating strong adhesion at microelectrodes on polyimide (PI) substrates. PI is a current attractive material for biodevices owing to its

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flexibility, chemical stability and biocompatibility. Pre-modification of the PI surface with self-assembled alkylsilane monolayers induced anisotropic lateral growth of PPy films along the substrate surface and enhanced film adhesion. Using a PPy-coated microelectrode, we have achieved reproducible, noninvasive, external stimulation of a cultured excitatory cell, namely the chick embryonic cardiac myocyte. The myocytes on the microelectrode substrate were electrically conjugated through gap junctions, and showed synchronized beating upon pulsation with the underlying PPy-coated microelectrode. The threshold charge required to stimulate an 0.8 cm^2 sheet of myocytes was estimated to be around 0.2 μC.

2. Experimental

Pyrrole (Kanto Chemical Co.) was purified by distillation under reduced pressure before use. Octadecyltrichlorosilane ($C_{18}SiCl_3$, Shinetsu Chemical Industries), polydimethylsiloxane (PDMS, KE-106, Shin-Etsu Chem. Co., Ltd.), fluo-3 AM (Molecular Probes, Eugene, OR), and all other chemicals were used without further purification.

Photosensitive PI (Toray, Photoneece) was spin-coated onto a glass plate (4000 rpm, 30 s), and baked at 150 °C for 90 min, resulting in a 2 μm thick PI film on the plate. A pair of microband electrodes was fabricated by photolithography with a sputter-deposited Pt film onto the PI-coated substrate. Each band-electrode was 20 μm wide and separated from the other by 10 μm, as illustrated in Fig. 1a. The electrode substrate was first treated with 2-propanol and then exposed to oxygen plasma (80 W, 1 min) for introduction of hydroxyl groups onto the PI surface. The substrate was then immediately immersed in a 0.2 mM $C_{18}SiCl_3$/heptane solution for 10 min to form a self-assembling monolayer of alkylsilane, followed by rinsing with pure heptane, ethanol and distilled water. A PDMS fence was used to set the electrochemical system on the substrate (Fig. 1a, b). The total electrode area exposed to the solution was ca. 1.6×10^{-3} cm^2.

The electropolymerization of pyrrole was conducted in an aqueous solution containing 0.1 M pyrrole and 0.1 M KNO_3 under potentionstatic conditions. Using a bipotentiostat (Fig. 1b), the polymerization potential for one of the two bands was set at 660 mV and the other at 680 mV vs. Ag/AgCl, as carried out in previous studies of PPy growth on a glass substrate [18]. Since the potentials of the two bands are slightly different, the ohmic currents are superimposed on the polymerization currents after electrical interconnection of bands with the growing PPy films. It is, therefore, possible to detect the moment of the interconnection by the ohmic current, and to evaluate the lateral growth rate across the gap of the electrode substrate.

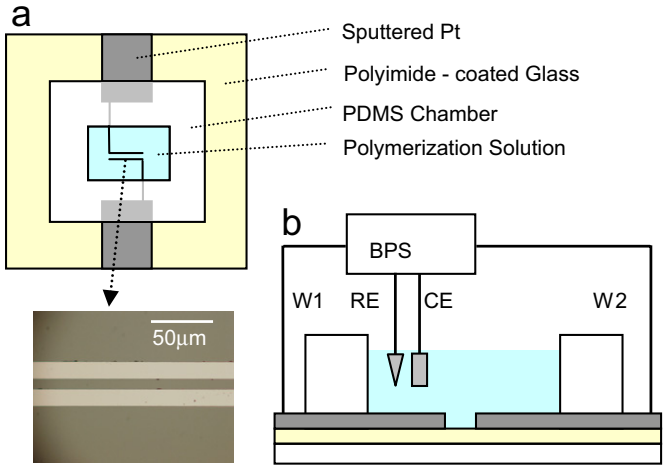

Fig. 1. (a) Schematic drawing of the Pt microelectrode substrate. A pair of Pt microband electrodes (20 μm width) were prepared on a polyimide-coated glass substrate. A PDMS chamber was placed over the substrate to define active electrode area to ca. 1.6×10^{-3} cm^2. (b) Electrochemical apparatus for electropolymerization of pyrrole at the microelectrode array with a bipotentiostat (BPS).

Primary cardiac myocytes were prepared according to a previously described method [16,17]. Cells were isolated from the hearts of chick embryos at Humburger and Hamilton stage 34–35 (8–9 days) by trypsinization and suspended in a culture medium consisting of 90% Dulbecco's modified eagle medium (DMEM) (Gibco), 10% fetal bovine serum (FBS) (Gibco), and a trace amount of penicillin–streptomycin (Gibco) solution. The cell suspension was pre-incubated in a tissue culture flask (Falcon) for 1 h to remove strongly adherent fibroblasts. The resulting myocyte-rich suspension (1×10^6 cells ml^{-1}) was seeded onto the PPy-coated microelectrode substrate. The cardiac myocytes attached onto the substrate surface were cultured in serum-containing medium to promote cellular spreading, growth and formation of cell–cell junctions.

Electrical stimulation of cardiac myocyte sheets was conducted with current pulses induced between the PPy-coated Pt microelectrode and the other Pt plate set in the culture chamber (Fig. 2), using an electronic stimulator (SEN-7203, Nihon Kohden) coupled with an isolator unit (SS-202J, Nihon Kohden). Intracellular Ca^{2+} imaging was performed during stimulation using a fluorescence microscope. Cultured myocytes were loaded with 10 μM fluo-3 AM for 30 min at 37 °C, and studied in a phosphate-buffered saline (PBS(+)) solution consisting of (mM) 0.90 $CaCl_2$, 2.68 KCl, 1.47 KH_2PO_4, 0.49 $MgCl_2$, 136.9 NaCl, 8.06 Na_2HPO_4, 5.55 glucose, and 0.327 sodium pyruvate (pH 7.4). FBS was removed during fluorescence measurements because it significantly increased background fluorescence and downgraded the S/N ratio. Cells were excited with irradiated light from a Xe lamp and fluorescence (excitation 488 nm/emission 530 nm) was detected with a digital CCD camera (HiSCA, C6790-81, Hamamatsu Photonics) through a barrier filter. Images were recorded on a computer and analyzed with Aquacosmos software (Hamamatsu Photonics).

3. Results and discussion

3.1. PPy growth at microelectrodes formed on PI substrates

Fig. 3a shows typical variations of the currents during polymerization at the twin-microband electrode on PI substrates. The solid and dotted line curves correspond to electrodes set at higher (680 mV) and lower polymerization

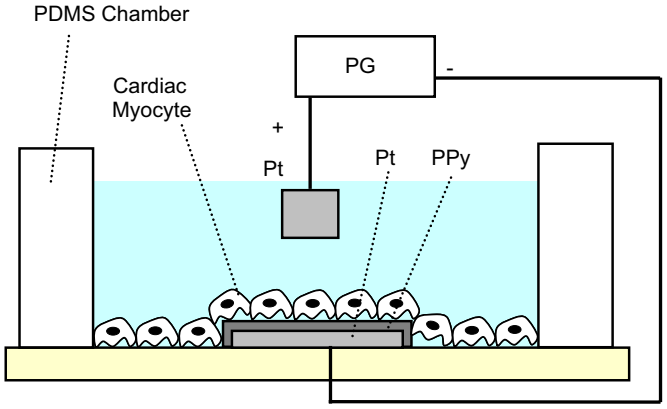

Fig. 2. Schematic diagram of the setup for electrical extracellular stimulation of the sheet of cultured cardiac myocytes with a current pulse generator (PG). Negative current was induced at the stimulation electrode.