Journal of Neuroscience Methods 213 (2013) 196–203

Contents lists available at SciVerse ScienceDirect

Journal of Neuroscience Methods

journal homepage: www.elsevier.com/locate/jneumeth

Basic Neuroscience

Photopatterning of self-assembled poly (ethylene) glycol monolayer for neuronal network fabrication

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HIGHLIGHTS

- Biomolecule patterning was realized by photolithgraphing chemical vapor deposited 2-[methoxy(polyethylenoxy)propyl] trichlorosilane.
- A patterned neuronal network was maintained for more than 3 weeks.
- Recorded neuronal networks activities showed high signal to noise ratio.

ARTICLE INFO

Article history:
Received 12 July 2012
Received in revised form
20 December 2012
Accepted 22 December 2012

Keywords:
Neuronal patterning
Chemical vapor deposited silanization
Poly (ethylene) glycol
Microelectrode arrays
Lithography
Neuronal activity

ABSTRACT

The ability to culture individual neurons and direct their connections on functional interfaces provides a platform for investigating information processing in neuronal networks. Numerous methods have been used to design ordered neuronal networks on microelectrode arrays (MEAs) for neuronal electrical activities recording. However, so far, no method has been implemented, which simultaneously provides high-resolution neuronal patterns and low-impedance microelectrode. To achieve this goal, we employed a chemical vapor-deposited, non-fouling poly (ethylene) glycol (PEG) self-assembled monolayer to provide a cell repellant background on the MEAs. Photolithography, together with plasma etching of the PEG monolayer, was used to fabricate different patterns on MEAs. No electrode performance degradation was observed after the whole process. Dissociated cortical neurons were cultured on the modified MEAs, and the patterns were maintained for more than 3 weeks. Spontaneous and evoked neuronal activities were recorded. All of the results demonstrate this surface engineering strategy allows successful patterning of neurons on MEAs, and is useful for future studies of information processing in defined neuronal networks on a chip.

1. Introduction

Neural patterning has attracted much interest in neural electronics (Jun et al., 2007; Jungblut et al., 2009; Offenhäusser et al., 2007) and neural engineering (Hynd et al., 2007). Compared with randomly cultured neurons, patterned neurons possess a much simpler network structure, which is easier for network modeling and signal analysis. In addition the amplitude of detected action potentials of neurons is larger if the locations of neurons are restricted directly on electrodes (Nam et al., 2004a).

With these advantages, different protocols have been developed to control neuron attachment and axon growth on substrates. Methods such as micro-contact printing (μCP) (Jun et al., 2007; Jungblut et al., 2009), photoresist processing (Chang and Sretavan, 2008), photodegradation (Baek et al., 2011) and microfluidics (Morin et al., 2006; Wang et al., 2009) have been applied for constraining neurons and axon guidance on substrates. By controlling the distribution of ligands suitable for selective adhesion of neurons on substrates, such methods are useful for constraining neurons and guiding neurites for forming patterned neuronal networks.

Abbreviation: MEAs, microelectrode arrays.

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http://dx.doi.org/10.1016/j.jneumeth.2012.12.020

However, the use of ligands for selective adhesion alone is not sufficient to provide the necessary repulsion of non-specific cell or protein attachment for effective long-term patterning (Chang and Wheeler, 2006). PEG polymers or PEG hyrdrogel have previously been used to solve this problem, since both ligand types render the substrates resistant to non-specific cell or protein attachment (Ichino and Nagasaki, 2006; Popat et al., 2003; Shah et al., 2008; Singhvi et al., 1994; Staii et al., 2009; Sugiura et al., 2008), making them suitable for long-term patterning (Branch et al., 2001). Silanization or thiol chemistry has mostly been implemented for fabricating PEG polymers or self-assembled monolayer on substrates (Jo and Park, 2000; Nam et al., 2004b; Singhvi et al., 1994; Staii et al., 2009). For silanes or alkanethiols, surface modification is performed either in anhydrous organic solution or through chemical vapor deposition (CVD) (Jönsson et al., 1985; Popat et al., 2003), and these self-assembled monolayers can be patterned through electrochemical desorption (Shah et al., 2008), electron beam etching (Zhang et al., 2005) or femtosecond laser exposure (Yamamoto et al., 2011). However, such surface modification of metal electrodes is known to increase the impedance of the metal electrodes (Nam et al., 2006), thereby increasing the noise level and reducing the likelihood of detecting minute signals.

Here, we present a novel technique that combines chemical vapor deposited silanization, photolithography and oxygen plasma etching to fabricate neuronal networks with a PEG background for preventing non-specific cell adhesion. Although PEG is not completely effective in preventing non-specific bio-molecule adhesion (Gref et al., 2000; Jo and Park, 2000), the application of lift-off techniques enables poly-D-lysine (PDL) as an anchor layer in our work for robust neuronal patterning and distribution. Our process of combining surface modification and photolithography allows to arbitrarily co-pattern adherence ligands and a non-fouling PEG monolayer on MEAs. This patterning technique has a stable effect of preventing non-specific cell adhesion and other biomolecules. In addition, the application of plasma etching is not only useful in patterning the PEG monolayer on the MEAs, but also removes any residues left on the micro-electrodes' surface. This technique reduces electrode contaminations and may therefore contribute to improving the signal to noise (S/N) ratio of detected action potentials. In this study, emphasis was placed on the applicability of this technique for fabricating neuronal networks on both custom built and commercial MEAs. Dissociated rat cortical neurons were deposited on the processed MEAs, and electrophysiological experiments were carried out to record the activities from patterned neuronal networks.

2. Materials and methods

2.1. PEG silanization

PEG patterning was performed on different substrates: silicon dioxide wafers, glass cover slips, custom built MEAs (PGI-8/ICS-8 Forschungszentrum Jülich), and commercial MEAs (60MEA200/30iR-ITO-w/o, MCS GmbH). All the substrates were resined in acetone, ethanol, and Milli-Q water for 15 min, 10 min, and 5 min, respectively, to remove any possible organic residues from the substrate surface. After drying in a nitrogen flow, the substrates were processed in a plasma chamber (O_2/Ar plasma at 1.4 mbar pressure and 200 W power) for 2 min to fabricate hydroxyl groups on the silicon dioxide surface. Then, the substrates were sealed in a desiccator together with 50 μL of 2-[methoxy(polyethylenoxy)propyl] trichlorosilane (MW range 470–610, Gelest, Inc.), in a glove box under an argon atmosphere. The pressure inside the desiccator was adjusted to 5 mbar by a vacuum pump, and the substrates were incubated for 1 h to deposit PEG self-assembled monolayers (SAMs) on substrate surface by CVD. After silanization, all these substrates were carefully removed from the glove box for further usage.

2.2. Contact angle measurements

Water contact angle measurements (Krüss, GmbH) were performed on the silicon dioxide wafers, which were divided into positive and negative control groups. Substrates in the negative control group were treated identically to that in the positive control group but without the silanization process. The contact angle of the substrates from the positive control group was assessed before and after silanization. After these tests, the substrates were processed by oxygen plasma and then assessed again. Three sample substrates were tested for every contact angle experiment, and each sample was tested at three different positions on the substrate surface ($N = 9$).

2.3. ATR-FTIR analysis

To confirm the existence of the self-assembled PEG monolayer on the substrate surface, ATR-FTIR was performed on the silicon dioxide wafers. The ATR-FTIR instrument is equipped with a liquid-nitrogen-cooled MCT detector. Spectra were recorded under N_2 at 4 cm^{-1} resolution and averaged over 256 scans to provide a relatively high signal-to-noise (S/N) ratio. Since silanization introduces methylene groups onto the substrate surface, the ATR-FTIR spectra were monitored over the scanning range from wavenumber 2700 cm^{-1} to 3100 cm^{-1}.

2.4. Photolithography and PDL patterning

A photoresist (AZ 5214) was spin-coated onto PEG-silane modified substrates. Standard photolithography was performed on the coated substrates using a quartz mask with chromium patterns. Patterns with nodes for cell soma attachment and lines for directing neurite outgrowth, have been used. According to the size of neuron soma and electrode, 30 μm nodes and 5 μm-wide lines were used for cell patterning. 24 μm nodes and 2 μm-wide lines were applied to pattern PLL-FITC. After developing in MIF326 developer (MIF326: DI water = 1:1, Hoechst) for 40 s, the photopatterned substrates were dried in nitrogen and processed in a plasma chamber for 5 min (O_2 plasma at 9 mbar, 200 W) to remove the unprotected PEG monolayer. For MEAs, 18 mm-diameter glass rings were glued on with PDMS to secure a region for subsequent cell culture. PDMS was cured in an oven for 1 h at 80 °C. With pattern PEG monolayer and photoresist on, these substrates were ready for further process.

2.5. Impedance measurement

The AC (alternating current) impedances at 25 different frequencies (1–10 kHz, 10 mVrms amplitude) were measured with a potentiostat/galvanostat (Autolab PGSTAT 30, Eco Chemie B.V., Utrecht, Netherlands) and a frequency response analyzer module (Autolab FRA2, Eco Chemie). For both custom and commercial MEAs, a single electrode of the MEAs was used as a working electrode, and an Ag/AgCl wire was used as a reference electrode. All measurements were performed in 1× PBS (phosphate buffered saline) solution at room temperature. The electrode impedances of the same five electrodes of each MEAs were measured after cleaning, after plasma etching, and after silanization. Average values and standard deviations of the impedances from each of the five electrodes were calculated.