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Hippocampal Neuronal Network Directed Geometrically by Sub-Patterns of Microcontact Printing (μCP)

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Abstract: The control of neuronal cell adhesion and growth in artificially defined networks in vitro was developed for the study of neuronal signals propagation. Microcontact printing (μCP) with PDMS microstamps was used as the chemical method to control cell adhesion and growth into defined networks. For controlled hippocampal neuronal cell growth, cytophilic poly-d-lysine (PDL) was patterned on glass substrates by μCP and then cytophobic PEG was self-assembled for passivation. The patterns were identified by fluorescent microscopy, atomic force microscopy, and condensation figure. The positively charged surface of PDL patterns interacted electrostatically with the negatively charged moieties of the cell membrane surfaces so that the attachment of the cells to the substrate surface was enhanced. The cultured hippocampus cells grew quite selectively along the printed PDL tracks. The track width suitable for forming single neuronal growth was discovered to be less than about 10 μm.

Keywords: *micro contact printing, self-assembly, neuronal cell growth, patterns, hippocampus*

Introduction

Researches on the interpretation of neuronal signals from a complicated excitable cell network and on the modification of the neuronal signals by using a chemical reagent or an electrical stimulus have been the major issues in the fields of physiology, neuroscience and basic medical science. The development of artificial neural networks in culture will allow a much higher resolution analysis at the electrical, metabolic, and structural level. It will make it feasible to perform analyses not possible in vivo [1]. It has become desirable and conceptually feasible to study small networks of synaptically interactive neurons in vitro. For this purpose, it is important to find the signal source in patterned neural networks with using planar multiple-electrode arrays (MEA) or field-effect transistor arrays (FET) [2].

It may be possible to discover the signal propagation algorithm in the neural network. Hence, neurons must be easily guided on the surface of the recording plate, survive along the artificially defined pattern and be monitored for sufficient time periods. The cell guidance along the defined patterns has been achieved using three different methodological categories. One is a topographical method which utilizes molds containing preformed wells [3]. The other is a chemical method which treats surface to express chemical functions [4]. Another is a cell-positioning technique which places a metal mask during the establishment of cell culture [5]. Chemical methods for surface patterning employ standard photolithography [4], deep UV lithography without the use of photoresist layers [6], and microcontact printing using elastomeric polydimethylsiloxane (PDMS) microstamps [2,7-11]. The PDMS microstamping method is simpler and more reusable compared to the others. Owing to its elastic and flexible characteristics, the former is versatile to treat various substrate surfaces including transparent glass slides that are adequate to observe the cell attachment through optical microscopy [2,8]. Combination of chemical functions to improve and control the selective neuron adhesion has been tried in various

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