Surface engineering techniques for designing neuronal networks *in vitro*

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By engineering a simple neuronal network *in vitro* that mimics the *in vivo* architecture and functions of the central nervous system (CNS), a novel system for studying of the dynamical properties of the CNS will become available. Although reconstructing the whole CNS circuitry is way beyond our expectation, we can still focus on its functional module, or local circuit, which consists of a small number of neurons. Neurons are the functional element of the CNS, and its circuitry is defined by the cell-types (glumatergic, GABAergic, etc.), neuronal polarity (axons and dendrites), and the site of synaptic contacts. Here we present our recent progress in developing surface nano/micro-modification techniques that enables us to address the latter two properties.

1. Directing axon-dendrite polarity of cultured neurons with surface micropatterns

The process of axon-dendrite polarity formation by cultured neurons has been well-documented [1]. Here, in an attempt to direct axon in an arbitrary direction, we investigated the role of a single neurite outgrowth in axon specification by extrinsically controlling lengths of individual neurites by culturing neurons on micropatterned substrates.

Embryonic rat hippocampal neurons were cultured on a micropatterned substrate of aminosilane and alkylsilane self-assembled monolayers (SAMs), cell permissive and non-permissive films, respectively, for 2 days. They were then fixed and stained for tau-1 (axonal marker) and MAP2 (somatodendritic marker) to evaluate polarization. Neurons that adhered to a pattern that allowed all neurites to elongate only 20 μm did not form an axon (n = 111 cells). Contrarily, when grown on a pattern that allowed a single neurite to extended up to 100 μm, neurons polarized, and 75% the neurite that grew on the 100 μm line was fated to become the axon (n = 145 cells). This result was also confirmed by live-cell imaging of transfected Kif5C^{560}, a constitutively-active form of Kinesin-1 (Kif5), which has been identified to translocate randomly among neurites before polarization and eventually accumulate stably in the axon after its specification [2]. Although the molecular mechanism of polarization in cultured neurons yet remains to be fully described, our results point out the requirement of differential elongation of future axon in neuronal polarization *in vitro*. Furthermore, we obtained a very simple method for selectively fating a neurite to differentiate in to an axon using surface micropatterns.[3]

2. Surface modification techniques for guiding individual neurites in vitro

After inducing axon formation, the next step in designing neuronal networks is to guide axons and dendrites of cultured neurons to a desired site to form circuits. For this, a method to modify cell-affinity of their scaffold in cell-culture environment is necessary. Conventional nanofabcrication techniques mostly process samples in air or vacuum and cannot be employed. A number of surface-modification methods have recently been proposed that are applicable even when substrate is immersed in liquid, and some enable μm-scale modifications [4]. Here we employed focused femtosecond (fs)-laser beam to ablate alkylsilane SAMs that prevented cell adhesion [5].

By scanning focused laser beam between aminosilane regions, on which primary chick forebrain neurons adhered and extended short neurites, the neurites were successfully guided and elongated along the laser-scanning line. Width of the laser-scanning line was evaluated by atomic force microscopy and was estimated to be 2.1 μm. The guidance was accomplished by multiphoton laser ablation of cytophobic SAM layer and subsequent adsorption of supplemented cell-adhesion molecule, laminin, onto the ablated region.

One drawback of the fs-laser processing was the low success rate of neurite guidance (~14%). To improve this, we are currently developing an alternative method that takes advantage of TiO_2 photocatalysis, whose experimental design and preliminary results will also be presented.

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