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Controlled extracellular topographical and chemical cues for acceleration of neuronal development

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ABSTRACT

Physical and chemical cues, which have emerged as a promising strategy for regulating cellular behaviors, provide important signaling cues to living cells. Neurons are also exposed to distinguishing physical and chemical environments that can greatly influence their behaviors and functions. In this study, we proposed the laminin-coated matrix nanotopography platforms (LMNPs) that generate extracellular physical and chemical cues for neuronal development. Using our platforms, we showed that nanotopographical and biochemical cues could provide suitable environments for neuronal cultures. More importantly, we showed that a LMNPs could control the orientation of neuronal structures as well as accelerate neuronal development through synergistic effects of extracellular nanotopographical and chemical cues. Our study imparts new design principles on the role of nanotopographical and chemical cues in neuronal development for the fabrication of neuroprosthetic scaffolds.

Introduction

The neuron is the basic cell of the central and peripheral nervous systems that enables the transmission of electrical activity to other neurons through the process of neuronal polarization. Therefore, understanding and controlling neuronal behaviors (e.g., neuronal development) is very important for overcoming neuronal injuries [1,2]. It is known that neurite outgrowth is strongly influenced by topographical and biochemical cues [3–7]. For example, engineered topographical platforms such as anisotropic topography (grooves and ridges) and isotropic topography (pillars and nanowires) can be used to provide effective environments for neuronal regeneration [8–11]. Additionally, Ketschek et al. demonstrated that poly-L-lysine- (PLL-) or laminin-coated surfaces can control the functions of myosin II and change the extension of axons, depending on the surface [12]. However, previous studies have mainly focused on the effects of topographical or biochemical cues solely for the purpose of understanding neuronal behaviors.

Here, we propose new engineering platforms of extracellular matrix (ECM) chemical components-coated matrix nanotopography that can generate both extracellular physical and chemical cues for neuronal development. We hypothesized that nanotopographical and chemical cues can synergistically affect neuronal behaviors. To this end, we first fabricated PLL-coated flat platforms, PLL-coated matrix nanotopography platforms (PMNPs), and laminin-coated matrix nanotogpography platforms (LMNPs) as cell culture platforms to provide controlled extracellular nanotopographical and chemical cues to neurons. Positively charged synthetic polymers, such as PLL, have been widely used as a coating material for neuronal cultures, and laminin is known as a substance present in the ECM that accelerates neuritic regeneration [13,14]. Interestingly, we showed that the LMNPs could control the orientation of neuronal structures as well as accelerate

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neuronal development through the synergistic effects of extracellular nanotopographical and chemical cues.

Materials and methods

Fabrication of neuronal culture platforms

Fig. 1(a) depicts the schematic procedure for fabricating the chemical and nanotopographical hybrid substrates. UV-curable poly(urethane acrylate) (PUA; Minuta Tech., Korea) with a photoinitiator was dropped on a nanopatterned silicon master mold (250–800 nm). The prepared mold was then covered with poly (ethylene terephthalate) (PET) film in a uniform thickness via capillary force. The mold was exposed to UV light (λ = 250–400 nm, 100 mJ/cm^2) for 15 s, after which the cured PUA replica was peeled off of the silicon mold using tweezers, and then again exposed to UV light for 10 h to annihilate any residual reactive acrylate groups. The nanopatterned PUA mold was then soaked in PLL and laminin solutions via dip coating for 15 s. Finally, the biochemical materials-coated nanoscale PUA substrates were fabricated after drying.

Neuronal cultures

Primary hippocampal neurons were cultured in serum-free conditions. The embryos were isolated from the pregnant rats at the 17th day of gestation, and then they were sterilizated in 1 mL of Hank's Balanced Salt Solution (HBSS) using a fire-polished Pasteur pipette. The suspended cells were centrifuged for 2 min at 1000 rpm to extract a cell pellet. The cell pellet was suspended in neurobasal media supplemented with B-27, 2 mM L-glutamine, 12.5 mM L-glutamic acid, and penicillin–streptomycin. Dissociated cells were seeded at a density of 50 cells/mm^2 on prepared substrates. Cultures were maintained in an incubator (5% CO_2 and 37 °C), and half of the media was replaced with fresh culture media without L-glutamic acid supplement every 3–4 days.

Adhered cells on the samples were fixed with 4% paraformaldehyde (Sigma–Aldrich, Milwaukee, WI, USA) for 20 min, permeabilized with 0.2% Triton X-100 (Sigma–Aldrich, WI, Milwaukee, USA) for 15 min, and stained with TRITC-conjugated phalloidin (Millipore, Billerica, MA, USA) for 1 h. Images of the stained cells were taken using a fluorescence microscope (Zeiss, Germany). A custom MATLAB script was used for quantitative analysis of the images.

Results and discussion

Design and fabrication of neuron culture platforms demonstrating extracellular nanotopographical and chemical cues

Various tissues and organs, including the brain, skeletal and muscular systems, and blood vessels, connect the central and peripheral nervous systems. In particular, the ECMs secreted by neurons and glia in the nervous tissues have complex topographical (e.g., aligned nanofibrils) and chemical (e.g., laminins and collagens) environments, providing specific cues to the neurons [15,16]. Topographical and chemical cues of ECMs have been thus known as important regulators of neuronal behavior, migration, and function (e.g., synapse formation) [17,18].

Based on these considerations, we designed chemical components-coated ECM-like nanopatterned substrates that produce both extracellular physical and chemical cues to neurons. To form the nanoscale grooved and ridged surfaces, we used e-beam lithography and UV-curable capillary force lithography (Fig. 1(a)). The aligned nanopattern was verified by scanning electron microscope (SEM), with size and pattern spacing of 250, 300, 400, 500, 600, 700, and 800 nm and a height of 400 nm. The fabricated nanopatterned platforms were then dip-coated with PLL and laminin. After drying, we checked the nitrogen concentration to confirm the amount of laminin on the substrate because laminin has an amine group (—NH_2). Larger amounts of nitrogen were found when the PLL and laminin were coated on PUA nanopatterns together compared to the PUA nanopatterns coated only with laminin. Fig. 1(d) represents the basic concept of this work: the structure and behavior of neurons guided by extracellular nanotopographical and chemical cues (e.g., controlled LMNPs). Consequently, we expected that the proposed hybrid platforms could control neuronal development by controlling neuronal structure.

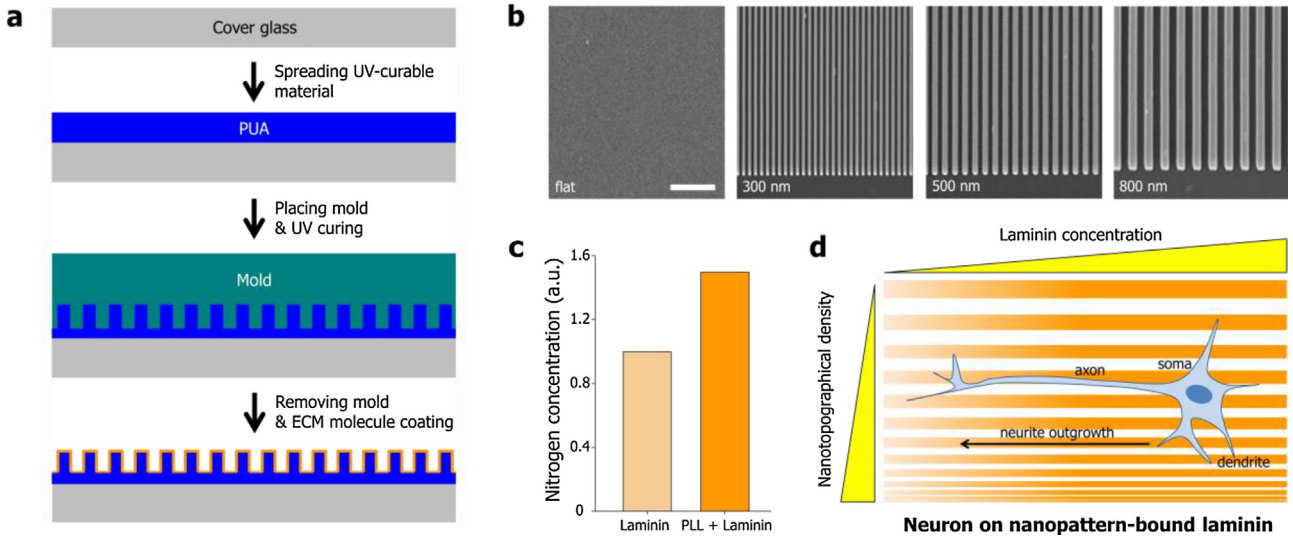

Fig. 1. Design and fabrication of nanotopographical and biochemical hybrid substrates as cell culture platforms for neurons. (a) Schematic of nanoscale-patterned PUA substrate with loaded biochemical cues. (b) Scanning electron microscope (SEM) images of unshaded nanotopographical substrates. White scale bars represent 5 μm. (c) The nitrogen concentration of laminin with or without PLL coated substrates. (d) Conceptual cell culture platforms for the guidance of behaviors of neurons with extracellular nanotopographical and chemical cues.

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