● PERSPECTIVES

A substrate scaffold for assessment of nerve regeneration and neurodegenerative diseases

Neuroregeneration is a complex topic in neuroscience and includes 3 concepts: neurogenesis, neuroplasticity, and neurorestoration. After injury of the nervous system, axons have the capacity for self-repair, regrowth or proliferation. The peripheral nervous system is more effective at restoring damaged axons than the central nervous system (CNS). This is because formation of scar tissue in the CNS influences neural regrowth or synthesis of growth-inhibiting proteins, thereby preventing reconstruction of a neural circuit (Silver and Miller, 2004; Enciu et al., 2011). Parkinson's disease (PD) and Alzheimer's disease (AD) are two most common degenerative diseases of the CNS among the elderly. PD is caused by death of dopaminergic neurons in the substantianigra, accompanied by aberrant aggregation of proteins termed Lewy bodies (LB) (Dauer and Przedborski, 2003). AD is also believed to involve a substantial loss of neurons in the hippocampus and some regions of the cerebral neocortex. Amyloid β (Aβ) is the major component of senile plaques and is one of the defining risk factors of neuronal cell death (Yankner, 1996). Nonetheless, the detailed pathogenesis of neurodegenerative diseases is unclear, and available treatments cannot effectively reverse progression of the neurodegenerative diseases.

Recently, many researchers attempted to combine the concepts of neuroregeneration and neurorestoration into an alternative therapy for neurodegenerative diseases. To address this issue, we need to know more about how neurons behave in theirnatural environment because most cells are surrounded by organs or tissues of varying stiffness ranging from approximately 100 Pa in the brain and fat to approximately 100,000 Pa in cartilage (Cox and Erler, 2011). Neuronal adhesion, neurite length, and mechanotransduction are mainly influenced by the extracellular matrix (ECM), which is often associated with structural scaffolding. Thus, studies of the above mentioned phenomena and the relation to substrate stiffness should provide new information aboutthe behavior of neurons. We have developed several culture environments, such as glass, plastic, and synthetic matrices, that closely mimic physiological growth environments (**Figure 1A**) (Chen et al., 2013).

The dorsal root ganglion neurons were cultured on the elastic substrate polydimethylsiloxane (PDMS) gel to study the mechanical forces acting on neurites. Through controlled indentation of individual neuritis by means of a glass pipette (*via* mechanical stimuli), we can induce an action potential. Furthermore, the mechanotransduction cascade is known to be directly affected by the cytoskeleton; therefore, we explored the cell structure and its effects. When we disrupted microtubules and actin filaments with nocodazole or cytochalasin D, respectively, the mechanically induced action potential was abrogated. In contrast, when we used blockers of channels such as transient receptor potential (TRP), acid-sensing ion channel (ASIC), and stretch-activated channels—while stimulating the cells mechanically—we observed almost no change in the firing of action potentials compared to mechanical activation of unmodified cells (Lin et al., 2009). We also assessed the relationship between substrate stiffness and outgrowth of hippocampal neurites by varying the ratio of the PDMS base to the curing agent to create substrates of varied stiffness. We changed elasticity of the PDMS substrate using the ratios 15:1, 35:1, and 50:1 to establish elastic moduli of approximately 173, 88, and 17 kPa, respectively, in order to model the growth environment in the brain.

The ECM is an important regulator of neuronal growth and function. ECM components such as collagen, laminin, and fibronectin act on surface membrane receptors to increase cell adhesion and neurite outgrowth. Therefore, we also compared effects of different ECMs on neuronal adhesion and neurite outgrowth. Immunostaining demonstrated that the hippocampal neurons exhibit greater neurite elongation on the 35:1 PDMS substrate compared to 15:1 PDMS, indicating that soft substrates provide more optimal stiffness for the hippocampal neurons. We also found that the hippocampal neurons exhibit more arborization during cell culture on a substrate coated with laminin. It is well known that cells can respond to environmental stiffness and other molecular cues during neurite extension, but few studies have explored the relevant signaling pathways. The

(A) A 2D PDMS-coated coverslip as an experimental model of a growth environment. (B) 3D neuronal culture mimicking the layered structure of the brain.
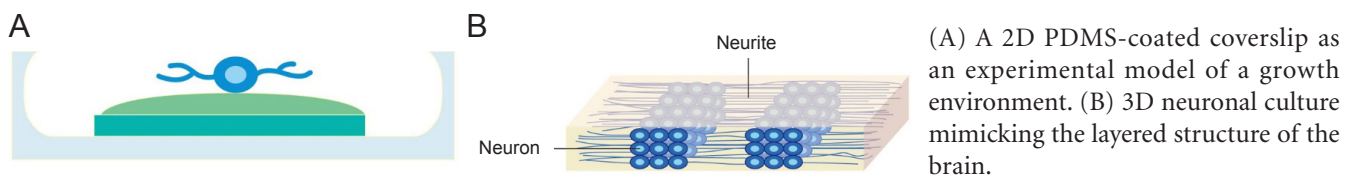
Figure 1 A schematic of the 2-dimensional (2D) or 3D culture system involving a polydimethylsiloxane (PDMS) substrate scaffold for measurement of neuronal growth.

hippocampal neurons exhibit improved attachment and neurite extension on substrates with specific stiffness (PDMS 35:1). Laminin and fibronectin can cause hippocampal neurons to elongate the neurites, and this process is accompanied by increased phosphorylation of FAK and ERK1/2 kinases compared to poly-L-lysine groups. These differences among signaling pathways point to crucial effects of substrate properties on regeneration of hippocampal neurons and neurite development. This work is expected to advance several scientific disciplines: neuroscience, biomaterials, and neuron-material interactions.

Growth of a neuron and communication of the neuron with the surrounding brain region develop in a three-dimensional (3D) space with complex and dynamic changes. In recent years, some research groups have used 3D substrates for neuronal culture to mimic the real environment of the brain more closely and to study neuronal networks, synapse plasticity, and neurodegenerative diseases such as Huntington's, Alzheimer's, or Parkinson's disease. Odawara et al. (2013) used the PDMS microchambers and collagen fiber orientation to mimic the layered structure of the brain (**Figure 1B**). 3D neuronal networks can control the soma position and the direction of neurite elongation, thereby closely mimicking the actual brain. Neural stem cells (NSCs) in 3D systems also follow a random migration pattern, extend longer neurites, and show different electrophysiological properties compared to 2D systems. A combination of optimal biomaterials and related techniques is expected to facilitate neuroregeneration and screening of candidate drugs for the treatment of neurodegenerative diseases. This approach should also shed light on the relevant neurophysiological mechanisms.

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