Using chips to simulate the brain as a tool to investigate brain development

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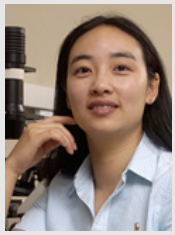

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"...microchip technology is very promising ... to facilitate our endeavor to cure nervous system diseases."

The brain is an extremely complex organ, with billions of neurons organized into different functional centers and each neuron making up to tens of thousands of connections with other neurons. The establishment of the intricate neuronal network involves many orchestrated steps during development, including neuronal stem cell (NSC) proliferation and differentiation, neuronal migration, axon and dendrite specification and guidance, and finally synapse formation. Despite enormous progress, the nervous system is still arguably one of the most difficult areas to study.

The fast advancement in microchip technology provides multifunctional platforms that mimic the integral *in vivo* environment yet retain the controllability of *in vitro* assays. Several micropatterning techniques have been developed to simulate the cell–extracellular matrix (ECM) and cell–cell interactions [1]. For example, microcontact printing (μCP) utilizes microfabricated polydimethylsiloxance devices as stamps to transfer the interested materials (as the ink) onto the substrate surface with desired patterns. The lift-off technology represents a different strategy, where the materials of interest are deposited onto the photoresist-patterned substrate. Subsequent lift-off of the photoresist will remove the materials on top of it and leave a pattern within the uncovered areas. Microfluidic channels or stencils have also been developed to pattern regions containing substrates. In addition to substrate patterning, microfluidic chip can deliver stimuli to cells via active fluid flow and control the spatial distribution of chemical cues. One widely used chip design is a pyramidal network of microchannels, which can create a smooth diffusible gradient in different shapes [2]. Another prototype is characterized by an H-shaped structure with a stable linear gradient generated within the central channel through diffusion. Different from the pyramidal network, the H-shaped design protects cells from active flow and alleviates sheer stress [3].

Compared with conventional approaches, microchip technology is capable of controlling the extracellular environment in a scale of cell size and delivering stimuli within milliseconds. In addition, this technology is more cost-effective and allows parallel experimentation, thus minimizing culture-to-culture variation. Applications of chip technology have contributed to the understanding of many biological questions such as cell fate regulation [4], bacteria biofilm formation [5] and gradient sensing of yeast [3] and neurons [6]. In this editorial, we will focus on how neurobiology research could benefit from these new techniques.

Neural stem cell proliferation, differentiation & specification in chips

Microenvironment or niche signals play important roles in regulating the differentiation and fate specification of NSCs [7]. Using microfluidic chips to generate a linear gradient of combined growth factors, Chung *et al.* examined the proliferation rate and fate determination of human NSCs in the presence of different absolute concentrations of growth factors [8]. Different from traditional in-dish cultures, the medium in the microfluidic chamber was constantly renewed in this study, thus the interfering autocrine and

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paracrine effects were minimized. In addition, the same batch of cells were exposed to different concentrations of growth factors within one experiment, greatly reducing the culture variation. How niche signals and cell-intrinsic factors interact and regulate NSC differentiation was also investigated in chips by immobilizing extracellular matrix components and morphogens, such as Wnt, bone morphogenic protein-4 and Notch [9]. Chips can be applied for single cell analysis as well. For example, neural precursor cells were cultured in a high-density array of micro-wells and physically isolated from one another. The constraint of cell movement allows simultaneous tracking of the fate of a large number (>3000) of individual cells over time [10]. In another study, Recknor and colleagues found that, when co-cultured with astrocytes on a substrate with both a microgroove pattern and laminin coating, adult hippocampal progenitor cells had a higher preference to acquire neuronal morphology than those cultured with astrocyte on a planar surface or those on grooves alone, demonstrating interactive effects from topographical, chemical and biological cues [11]. Therefore, the application of microchip technology provides a more faithful simulation of the interactions between NSCs and the environment, which is not only important for basic understanding of the stem cell biology, but may also shed light onto the design of stem cell-based therapies for treating neurological diseases.

Neuronal migration & axon guidance in chips

Neurons and their axons and dendrites are guided to their targets by either local or long-range factors in the brain [12]. One of the early attempts to use microchip technology to study axon guidance was performed by Clark *et al.* [13]. They showed that the fidelity of neurites' alignment with micropatterned laminin stripes depends on the stripe geometry and on the neuronal types. Topographic map formation in the visual system requires gradients of both guidance factor Ephrin and its receptor Eph [14]. Using μCP to generate a surface-bound gradient of Ephrin, Philipsborn *et al.* demonstrated that growth cones from chicken temporal retinal neurons integrate both the local concentration and the accumulative amount of Ephrin they encounter with to determine their stop zones within the gradient [15]. Several families of guidance cues are shown to form diffusible gradients that function in long distances to influence the growth cone directionality [16]. To precisely control the shape and the onset of diffusible gradients, an improved microfluidic chip has been developed with several pyramidal networks of microchannels and integrated in-chip valves [6]. Using this system, the guidance of axons from *Xenopus* spinal neurons was examined in composite N-shaped substrate-bound laminin gradient and a linear diffusible gradient of brain-derived neurotrophic factor. This study represents the first direct demonstration that ECM molecules and guidance factors can synergistically tune the directional decision made by axons during navigation.

Investigation on neuronal migration *in vitro* has been using the traditional micropipette system [17,18] or Boyden chamber assay [19]. One of the drawbacks of these approaches is that the guiding signals cannot be finely controlled. The microfluidic platform has been used extensively in studying chemotaxis of bacteria, yeast, amoeba and leucocytes [3,20–22]. In principle, microfluidic chips can also be applied to study neuronal migration. The initial application was hurdled by the difficulty of maintaining healthy neurons in a fluidic environment owing to neurons' extra sensitivity to sheer stress. Recent success of culturing neurons in chips while limiting the sheer stress thus makes the chip technology friendly for studies on neuronal migration [6].

Axon-dendrite specification in chips

Unlike many other cell types, neurons are polarized cells; with dendrites to receive inputs while axons to send out signals. Both intrinsic and extrinsic signals have been shown to regulate this process [23]. Using a microfluidic device to generate alternating stripes of two of the three ECM proteins: poly-L-lysine, laminin and neuron-glia cell adhesion molecules (NgCAM), Esch *et al.* examined axon specification of rat hippocampal neurons [24]. Interestingly, axons are preferentially formed on laminin or NgCAM. In another study, hippocampal neurons were cultured on graded laminin created by a microfluidic chip. The longest neurites of these neurons are found to be facing the direction with increasing laminin concentration, and this preference is correlated with the steepness of the gradient [25]. Geometric constraints such as the dimension and the shape of 3D structures also appear to regulate the specification of axons [26]. These studies provide valuable information on how the extracellular environment regulates the development of neuronal polarity.

Synaptogenesis & neuronal network formation in chips

While electrophysiological recordings of neurons using traditional micropipettes have contributed tremendously to our understanding of neuronal functions, how the vast neuronal network functions as a system cannot be easily studied by this approach. The microbiosensor formed by multi-electrode arrays (MEAs) can detect the signal of many neurons in a network simultaneously, with high sensitivity and in real time. MEAs can also be used to detect the released neurotransmitter during a synaptic event via sensing the current generated by the chemical reaction on the electrode surface [27,28]. Incorporation of MEAs with other microelectromechanical systems has been applied to measure not only the electrical activity, but also other responses such as pH and temperature in a neuronal network under high-frequency electromagnetic fields [29].

Micropatterning techniques can facilitate the studies on synaptogenesis. For example, by surface patterning agrin using covalent μCP, Cornish *et al.* demonstrated how this motoneuron-derived molecule could induce the accumulation of acetylcholine receptors in the postsynaptic muscle cells [30]. Surface

modification combined with MEAs has also been developed to investigate the electrical activities within neuronal networks [31–34]. In these studies, patterns of adhesion molecules were aligned with MEAs, physically confining cell bodies with electrode sites and guiding the neurites to form defined connections with neighboring neurons. Besides chemical manipulation of the substrate, neurons can also be patterned using negative dielectrophoretic forces [35] or microfluidic devices [36]. The integration of microfluidic system, μCP for surface patterning and MEAs for recording enables directed delivery of neurotransmitter to the engineered neural network, constituting a so-called artificial synapse [34]. The activity of neural network can be examined both electrically and chemically using this system. Future development of more sophisticated chips may broaden our understanding on how our brain functions as a whole.

Conclusions

Microchip technology has been applied to study almost every subfield of neurobiology. The research on NSC behavior and axonal specification has both benefited from protein or cell patterning using surface modification techniques. Microfluidic gradient generators will be especially useful in studying stem cell migration, neuronal migration and axon guidance. The utilization of MEAs allows scaled-up and detailed analysis of behaviors of neurons within a network. By integrating these multidisciplinary techniques, microchip technology is very promising to reveal new principles governing brain development and function and thus to facilitate our endeavor to cure nervous system diseases.

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References

1 Park TH, Shuler ML. Integration of cell culture and microfabrication technology. *Biotechnol. Prog.* 19, 243–253 (2003).

2 Jeon NL, Dertinger SK, Chiu DT *et al.* Generation of solution and surface gradients using microfluidic system. *Langmuir* 16, 8311–8316 (2000).

3 Paliwal S, Iglesias PA, Campbell K *et al.* MAPK-mediated bimodal gene expression and adaptive gradient sensing in yeast. *Nature* 446, 46–51 (2007).

4 Chen CS, Mrksich M, Huang S, Whitesides GM, Ingber DE. Geometric control of cell life and death. *Science* 276, 1425–1428 (1997).

5 Cho H, Jonsson H, Campbell K *et al.* Self-organization in high-density bacterial colonies: efficient crowd control. *PLoS Biol.* 5(11), 2614–2623 (2007).

6 Wang CJ, Li X, Lin B *et al.* A microfluidics-based turning assay reveals complex growth cone responses to integrated gradients of substrate-bound ECM molecules and diffusible guidance cues. *Lab. Chip* 8, 227–237 (2008).

7 Ming G-l, Song H-J. Adult neurogenesis in the mammalian central nervous system. *Annu. Rev. Neurosci.* 28, 232–250 (2005).

8 Chung B, Flanagan L, Rhee S *et al.* Human neuronal stem cell growth and differentiation in a gradient-generating microfluidic device. *Lab. Chip* 5(4), 401–406 (2005).

9 Soen Y, Mori A, Palmer TD, Brown PO. Exploring the regulating of human neural precursor cell differentiation using arrays of signaling microenvironments. *Mol. Syst. Biol.* 2, 37–50 (2006).

10 Chin VI, Taupin P, Sanga S *et al.* Microfabricated platform for studying stem cell fates. *Biotech. Bioeng.* 88(3), 399–415 (2004).

11 Recknor J, Sakaguchi D, Mallapragada S. Directed growth and selective differentation of neural progenitor cells on micropatterned polymer substrate. *Biomaterials* 27(22), 4098–4108 (2006).

12 Tessier-Lavigne M, Goodman CS. The molecular biology of axon guidance. *Science* 274(5290), 1123–1133 (1996).

13 Clark P, Britland s, Connolly P. Growth cone guidance and neuron morphology on micropatterned laminin surfaces. *J. Cell Sci.* 105, 203–212 (1993).

14 Drescher U, Bonhoeffer F, Muller BK. The Eph family in retinal axon guidance. *Curr. Opin. Neurobiol.* 7(1), 75–80 (1997).

15 Philipsborn ACV, Lang S, Loeschinger J *et al.* Growth cone navigation in substrate-bound ephrin gradients. *Development* 133, 2487–2495 (2006).

16 Kim JY, Ming G-l. Adaptation mechanisms in long-range growth cone guidance. *Cellsci. Rev.* 4(4), 97–109 (2008).

17 Guan C-b, Xu H-T, Jin M, Yuan X-B, Poo M-M. Long-range Ca^{2+} signaling from growth cones to soma mediates reversal of neuronal migration induced by slit-2. *Cell* 129(2), 385–395 (2007).

18 Xu H-T, Yuan X-B, Guan C-B *et al.* Calcium signaling in chemorepellant slit2-dependent regulation of neuronal migration. *Proc. Natl Acad. Sci. USA* 101(12), 4296–4301 (2004).

19 Hassoun AT, Erdelyi F, Szabo G, Davis MI. A rapid screening method for population-specific neuronal motogens, substrate and associated signaling pathways. *J. Neurosci. Methods* 166(2), 178–194 (2007).

20 Mao H, Cremer PS, Manson MD. A sensitive, versatile microfluidic assay for bacterial chemotaxis. *Proc. Natl Acad. Sci. USA* 100(9), 5449–5454 (2003).

21 Lin F, Nguyen CM-C, Wang S-J *et al.* Effective neutrophil chemotaxis is strongly influenced by mean IL-8 concentration. *Biochem. Biophys. Res. Commun.* 319, 576–581 (2004).

22 Song L, Nadkarni SM, Bodeker HU *et al.* Dictyostelium discoideum chemotaxis: threshold for directed motion. *Eur. J. Cell Biol.* 85, 981–989 (2006).

23 Yoshimura T, Arimura N, Kaibuchi K. Signaling networks in neuronal polarization. *J. Neurosci.* 26(42), 10626–10630 (2006).

24 Esch T, Lemmon V, Banker G. Local presentation of substrate molecules directs axon specification by cultured hippocampal neurons. *J. Neurosci.* 19(15), 6417–6426 (1999).

25 Dertinger S, Jiang X, Li Z, Murthy V, Whitesides G. Gradients of substrate-bound laminin orient axonal specification of neurons. *Proc. Natl Acad. Sci. USA* 74(11), 2451–2457 (2002).

26 Francisco H, Yellen BB, Halverson DS, Friedman G, Gallo G. Regulation of axon guidance and extension by three-dimensional constraints. *Biomaterials* 28, 3398–3407 (2007).

27 Bogorff DJ, Messerli MA, Malchow RP, Smith PJ. Development and characterization of a self-referencing glutamate-selective micro-biosensor. *Biol. Bull.* 205, 207–208 (2003).

28 Crespi F. *In vivo* voltammetry with micro-biosensors for analysis of neurotransmitter release and metabolism. *J. Neurosci. Methods* 34, 53–65 (1990).

29 Koester P, Sakowski J, Baumann W, Glock H-W, Gimsa J. A new exposure system for the *in vitro* detection of GHz field effects on neuronal networks. *Bioelectrochemistry* 70, 104–114 (2007).

30 Cornish T, Branch D, Wheeler B, Campanelli J. Microcontact printing: a versatile technique enhances polylysine transfer and neuronal cell patterning. *Mol. Cell. Neurosci.* 20(1), 140–153 (2002).

31 Nam Y, Chang J, Wheeler B, Brewer G. Gold-coated microelectrode array with thiol linked self-assembled monolayers for engineering neuronal cultures. *IEEE Trans. Biomed. Eng.* 51(1), 158–165 (2004).

32 James CD, Spence AJ, Dowell-Mesfin NM *et al.* Extracellular recordings from patterned neuronal networks using planar microelectrode arrays. *IEEE Trans. Biomed. Eng.* 51(9), 1640–1648 (2004).

33 Chang J, Brewer G, Wheeler B. Modulation of neural network activity by patterning. *Biosens. Bioelectron.* 16(7–8), 527–533 (2001).

34 Peterman MC, Mehenti NZ, Bilbao KV *et al.* The artificial synapse chip: a flexible retinal interface based on directed retinal cell growth and neurotransmitter stimulation. *Artif. Organs* 27(11), 975–985 (2003).

35 Yu Z, Xiang G, Pan L *et al.* Negative dielectrophoretic force assisted construction of ordered neuronal networks on cell positioning bioelectronic chips. *Biomed. Microdevices* 6(4), 311–324 (2004).

36 Morin F, Nishimura N, Griscom L *et al.* Constraining the connectivity of neuronal networks cultured on microelectrode arrays with microfluidic techniques: a step towards neuron-based functional chips. *Biosens. Bioelectron.* 21, 1093–1100 (2006).