Improved culture of human stem cell neurons in XonaChips™
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XonaChips™

Abstract
The XonaChip™ offers advantages for culturing neurons differentiated from human stem cells. Differentiated neurons attach and distribute more evenly with the XonaChip™ than in silicone-based devices, resulting in healthy cultures that can be maintained for 4-5 weeks or more.

Introduction
Neurons differentiated from human stem cells are increasingly used in neuroscience. The unique extreme polarization of these and other post-mitotic neurons demands an approach to measure and manipulate distinct neuronal compartments. Microfabricated multicompartment devices, pioneered by Xona scientists, have become well-established and well-used research tools for neuroscientists in the last 10-15 years. These devices compartmentalize neurons and provide a method to physically and chemically manipulate subcellular regions of neurons, including somata, dendrites, axons, and synapses.

To provide an easy-to-use and fully assembled device, Xona has now developed plastic XonaChips™ (see Introducing XonaChips™ for more details). In this TechNote, researchers at UNC-Chapel Hill differentiated human neural stem cells into glutamatergic neurons and found that XonaChips improve the long-term culture of these neurons over previous silicone-based compartmentalized devices.

Results & Discussion
Human NSCs differentiate into neurons and neuronal projections enter the axonal compartment after one week (7-10 days) in the chip with differentiation media (Figure 1). The resulting neurons within the chip attach and distribute more evenly within the somatic compartment than in PDMS devices. Neurons in the XonaChips™ had healthy bundled axons and neurons can be maintained within the chips for 4-5 weeks.

Methods
XonaChips™ were prepared according to the protocol. XC Pre-Coat™ was first used to ensure even wetting of the chip. XC Pre-Coat™ is included with each XonaChip™ order. The chip was then coated with Poly-L-Ornithine (20 µg/ml) and laminin (10 µg/ml) before pre-conditioning with NSC media. Poly(dimethylsiloxane) (PDMS) or silicone-based compartmentalized devices were prepared according to Xona’s silicone-based devices protocol. These devices were also coated w with Poly-L-Ornithine and laminin before pre-conditioning with NSC media.

Figure 1. Human stem cell differentiated neurons grown in XonaChips™ and side-by-side comparison with PDMS compartmentalized devices. (A) A phase contrast image of human neurons grown at 13 days after differentiation in XonaChips™. (B) A zoomed in region of the neurons cultured within the white box in (A) and an equivalent region within a PDMS compartmentalized device (right). hSC-neurons within the chips attach well and remain evenly distributed throughout the culturing period. Aggregated neuron clusters form in PDMS-based devices after several days in culture. Representative of >5 experiments. Images were acquired by Dr. Smita Paranjape (UNC-Chapel Hill).
H9-derived human neural stem cells (ThermoFisher Scientific, 510088) were thawed according to the manufacturer’s instructions. Approximately 70,000 NSCs were seeded into the right compartment of the XonaChip™. The same number were seeded into the right compartment of the PDMS compartmentalized device. Images were acquired with an inverted phase contrast microscope.

**Conclusion**

In summary, the XonaChip™ is a fully assembled multicompartment device that is easy to use and results in healthy, long-lasting cultures. Importantly, as shown previously these chips allow microenvironments to be established as with our SND series devices and are equally compatible with high-resolution fluorescence microscopy.

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**About Xona Microfluidics, LLC**

Xona Microfluidics, LLC is a California LLC based in Temecula, California with R&D facilities in Research Triangle Park, North Carolina. More information can be found at xonamicrofluidics.com.

If you are interested in testing a XonaChip™ contact us at info@xona.us

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