Retrograde labeling of neurons using pseudorabies virus in XonaChips®

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Abstract

This TechNote describes fluorescent retrograde labeling of neurons via pseudorabies viral infection of axons isolated within the axonal compartment of XonaChips®. Results using this method are illustrated using E18 rat hippocampal neurons and similar results are found using human stem cell derived neurons.

Introduction

This TechNote describes a procedure for retrograde labeling of neurons within XonaChips® using G-deleted rabies virus. Retrograde tracers such as G-deleted rabies virus expressing fluorescent proteins (incompetent for trans-synaptic transfer) are used to identify neurons that project their axons into the axonal compartment. Pseudorabies virus also retrogradely labels neurons *in vivo* to trace neuronal circuitry[1]. Using this virus we labeled both human and murine derived neurons in compartmentalized microfluidic devices[2-7]. Importantly, fluorescence labeling of neurons with this virus helps to visualize the entire dendritic tree, including fine structures such as filopodia and spines.

In addition, several other viruses have been used in Xona Microfluidic' devices including Alpha-Herpesvirus, Herpes Simplex Virus 1, Enterovirus, and, Human Coronavirus to study their axonal transport and spread within neurons [8-12]. Hence, these microfluidic devices provide a novel method for understanding the route of viral infections and how they modulate neuronal function in CNS.

For more information on cyclic olefin copolymer XonaChips® [4,6], see the Introducing XonaChips® technote.

Methods

Neurons should be first cultured within XonaChips® for at least 1 week or until sufficient axonal growth is present in the axonal compartment before proceeding with labeling steps below. Refer to the appropriate XonaChip® protocol for details.

NOTE: G-deleted rabies virus was purchased from GT3 Core Facility of the Salk Institute by University of North Carolina at Chapel Hill[4,6] in collaboration with Xona Microfludics, Inc. Handle potentially infectious materials according to the local organization's guidelines. Additional training may be required.

1. Warm up fresh neuronal culture media to 37 °C. Use approximately 400 µl to 500 µl of media per chip.
2. Remove 50 µl of media from either well of the axonal compartment and store in a 1.5 ml centrifuge tube.
3. Add 100,000 viral units of the selected modified rabies virus to the 1.5 ml centrifuge tube from step-2.

NOTE: Tips and tubes in contact with the virus need to be disposed according to your organization-approved protocol.

4. Gently remove the remaining media from the wells of the axonal compartment into a centrifuge tube and store it at 37 °C.

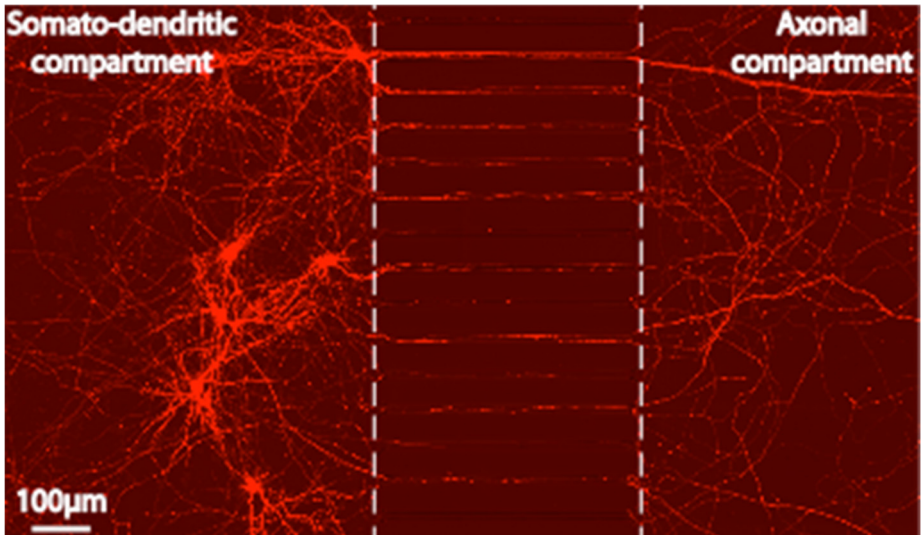

Fig. 1: Retrograde labeled neurons express mCherry fluorescent protein. (A) A merged fluorescence micrograph showing live retrograde labeled neurons infected via a modified mCherry rabies virus briefly applied to the axonal compartment. Neurons were imaged 3 days post-infection at 21 days in culture.

5. Add 150 µL of fresh warm media with the diluted virus to the axonal compartment. Place the XonaChip® in the incubator for 2 h at 37° C.
6. After incubation, gently remove and properly dispose of the virus-containing media from the axonal compartment.

NOTE: Air bubbles may become trapped in the chip if fluid is vigorously removed or aspirated from the main channels.

7. Gently add 75 µL of fresh media to one axon well and allow it to flow through to the connecting axonal well.
8. Remove and dispose properly the flow-through from the second axonal well.
9. Repeat steps 7 and 8 once. Add reserved media from step 4 to the axonal compartments. Add approximately 50 µL fresh media, if necessary, to maintain adequate volume and return the cells to the incubator.

Results

E18 rat hippocampal neurons were cultured within two compartment XonaChips® (XC450) coated with XC Pre-Coat and XC PDL as described in the XonaChip® protocol. We retrogradely labeled neurons that extended axons into the axonal compartment by applying a G-deleted mCherry rabies virus (incompetent for trans-synaptic transfer) to the axonal compartment at 18 days *in-vitro*. Neurons were imaged 3 days post-infection (21 days *in-vitro*). Montages of fluorescently labeled live neurons were captured within the chip to visualize the entire neuron including dendrites in the somato-dendritic compartment and axons that extend through the microgrooves into the axonal compartment (Fig. 1). Images were acquired with a laser scanning Confocal microscope using a 30×/1.05 N.A. silicone oil (ne = 1.406) objective lens.

Conclusion

In summary, neurons can be easily retrogradely labeled via viral infection within both Xona's Silicone Devices and XonaChips®.

About Xona Microfluidics, Inc.

Xona Microfluidics, Inc is a life sciences company based in Research Triangle Park, North Carolina. More information can be found at xonamicrofluidics.com.

If you are interested in testing XonaChips® contact us at info@xona.us

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