Monitoring Primary Neuron Growth and Development on Microruled Cell Culture Plates: Evaluation Utilizing Cryopreserved Rat DRG Neurons

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Nervous system function is dependent on the highly specific neuron–neuron interaction and connections established during development. The initial steps in this process involve neurite extension toward the proper targets guided by the growth cone in response to environmental signals. Evaluating neurite outgrowth, patterning, and specificity of these connections during development and under conditions of pharmacologic (neurotropic and neuroprotective) or environmental stress is best undertaken in primary cell culture. For these studies, the counting of cells, neurite branching, and neurite elongation over time need to be accurately monitored and measured. Such studies require precision.

Subtle changes in neuronal cell number during growth and during exposure to nutritive or trophic influences and following drug or toxin challenge are best assessed quantitatively. Automated fluorescent-based microscopic or plate-based analysis, while giving general estimations of cell populations, are not sensitive or precise enough to determine how the cells distribute themselves within a culture plate/well, or for proper evaluation of cell anatomy and development. This is best done using microscopic evaluation of individual primary neuronal cell cultures. This has previously required the use of eyepiece graticules, and analysis was often biased depending on the user and positioning of the culture in the microscope, since there is no absolute indication mark on the cell culture surface.

Dissociation and production of rat dorsal root ganglion (DRG) cells for culture is labor intensive and offers low yield. As such, DRG cells have been considered unsuitable for use in multiple-well format assays.

In this paper, the use of commercially available cryopreserved rat DRG cells (**QBM Cell Science Inc.,** Ottawa, Canada) cultured on the microruled Cellattice™ coverslip and 96-well plate (**Nexcelom Bioscience,** Lawrence, MA) is described.

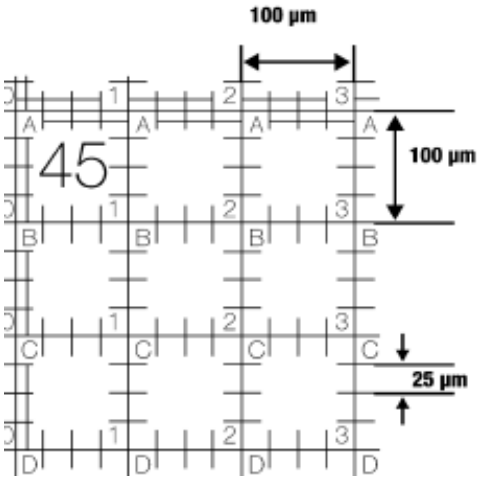

Figure 1 Illustration of Cellattice marking system.

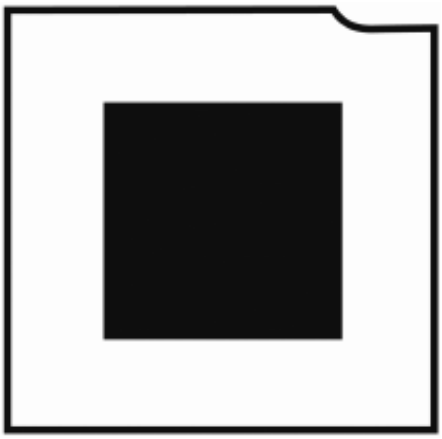

Figure 2 Schematic of the Cellattice coverslip.

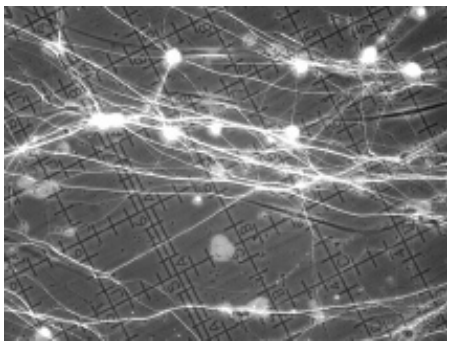

*Figure 3 Cryopreserved dorsal root ganglion cells (**QBM Cell Science**) dissected from postnatal rat pups day 2–3. Cells were grown for 26 days on a sterile Cellattice coverslip with a microruled cell culture surface. Prior to plating, the coverslips were coated with PDL laminin.*

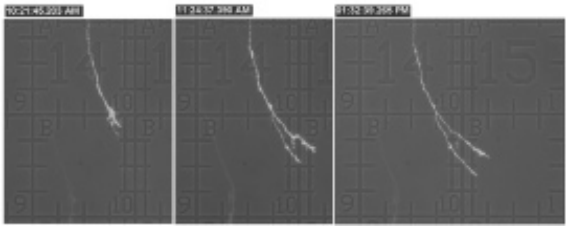

*Figure 4 Cryopreserved DRG cells were plated on Cellattice coverslips coated with PDL laminin. At day 1 (day after plating), cells were transfected with TransMessenger™ transfection reagent (**Qiagen Inc.,** Valencia, CA). Pictures were taken showing axonal outgrowth at different time points. Growing the cells on Cellattice coverslips allowed for an easy way to locate cells of interest and instant measurement of the length of the axonal growth between different time points.*

Cellattice is an optically smooth plastic with microscopic identification and measurement markers. It is fabricated into coverslips, 24-well plates, and 96-well plates. *Figure 1* illustrates the Cellattice marking system. The combination of numbers, letters, and tick marks identifies each 25 µm in a 10 × 10 mm area for the coverslip. The notch position indicates the cell culture surface (*Figure 2*).

Rat DRG cells (nonpurified) were isolated and dissociated at P1 using standard procedures and then cryopreserved. They were then thawed and plated at 5K per well on poly-D-lysine (PDL)-coated Cellattice 96-well plates and grown in neurobasal medium with B27. No further supplementation was required for survival to 28+ days.

The DRG neurons were examined using immunohistochemistry for neuron-specific β-tubulin (*Figure 3*). There was an abundance of long, loosely bundled axons within the cultures that followed the pattern of the underlying Schwann cells. Over the first few days, neuronal cell bodies formed ganglionated clusters and fasiculated axon bundles, but with the addition of mitotic inhibitors to limit glial cell division, DRG neurons and their axons remained dispersed. These cultures were superior for most types of study and, in fact, cultures not treated with mitotic inhibitors became useless at approx. day 12 due to detachment of ganglionated clusters from the substrate.

Within 2–3 days in culture, the cryopreserved DRG neurons displayed extensive neurite outgrowth, and by day 10 showed the same size and neurochemical type distribution as freshly dissociated rat DRG neurons. This is consistent with the generally accepted view that, after 9 days in culture, DRG neurons are physiologically mature. DRG neurons have been generally classified into two populations on the basis of cell size and morphology, with an overlap in the size distribution for these populations. The large neurons (typically mechanoreceptive and thermoreceptive A-type sensory cells) and small neurons, which are polymodal nociceptors, were evident for the cultured cryopreserved DRG neurons (Figure 1). The DRG cell axon outgrowth and development could be followed against the microruled cell culture surface, and the outgrowth of fluorescent processes could be readily imaged (*Figures* 3 and 4). Using recurrent digital imaging, the cell soma, proximal and distal axon, growth cone, and fine filopodia of the pseudounipolar neurons with branched neurites were easily monitored over time (Figure 4). Each growth cone displayed multiple lamellipodia, all with normal length and morphology.

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