Application Note

Damage-free sorting of DRG neuron cells responsible for pain transmission

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Introduction

In recent years, research on neural activity related to the pain and development for the visualization of pain has been carried out in various fields. The cell body of a sensory neuron (afferent neuron) called DRG neuron found in Dorsal Root Ganglions (DRGs) is responsible for the pain transmission from outside world and organs. However, there are many other types of cells that exist inside DRGs, and thus they consist of heterogeneous cell populations. Depending on the DRG neuron types that exist within the DRGs, the cellular response to pain is known to be different. Therefore, isolating a specific type of DRG neuron from a heterogeneous sample allows researchers to get a better understanding on its biochemistry and electrical signal on neurotransmission, which could be relevant for drug development. As On-chip Sort has eliminated all the damaging steps involved in sorting on conventional sorters, sorting of fragile cells such as DRG neurons is made possible. This application note presents the capability of On-chip Sort to isolate (1) DRGs by size and (2) DRGs with high expression of isolectin B4 (IB4) binding proteins on the cell surface.

Methods

1. Sorting of DRGs by size

DRGs collected from a 10-week old adult rat were obtained, dissociated by enzyme treatment, and suspended in Neural Basal Medium (with 10% FBS and 1% NGF added) prior to sorting. The pre-treated sample was sorted according to size (Fig. 1a) and cultured for five days. Sorted cells were fixed by paraformaldehyde, and stained with anti-β-tubulin III, which recognizes microtubule protein found in neurons, and anti-Synaptophysin, which recognizes neurons responsible for neurotransmission.

2. Sorting of IB4-positive DRG neurons

Heterogeneous DRGs collected as per above were stained with Alexa546-conjugated anti-IB4 for 30 min, washed with medium, and observed under a fluorescence microscope. Stained DRG cells with high IB4 expression were isolated by On-chip Sort, and then cultured for four days.

Results

1. Sorting of DRGs by size

Four groups of cells were observed from the size distribution, and two of these cell populations were separately isolated on On-chip Sort (Fig. 1a). and cultured for five days. Both populations were fixed by paraformaldehyde and stained with anti-β-tubulin III and anti-Synaptophysin (Fig. 1b).

Successful staining by anti-β-tubulin III and anti-Synaptophysin staining, the two isolated cell populations were confirmed to be viable nerve cells in DRGs after five days of culture. These results reveal that On-chip Sort is capable of isolating DRG neurons by size while maintaining viability.

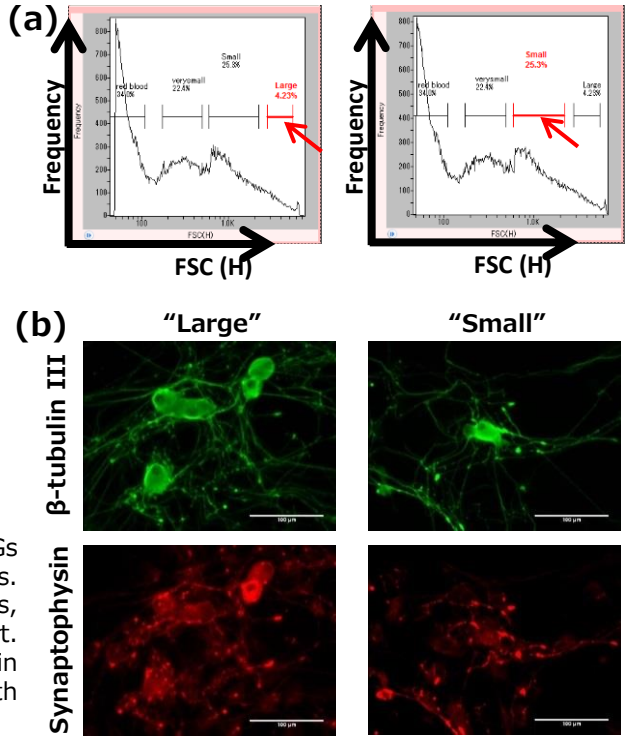

Fig. 1. (a) FSC histograms of a heterogeneous DRGs sample on On-chip Sort showing size distribution of cells. Two separate cell populations (indicated by red arrows, named 'large' and 'small') were isolated on On-chip Sort. (b) Images of isolated 'large' and 'small' cell populations in (a), cultured for five days, and confirmed by staining with anti-β-tubulin III (green) and anti-Synaptophysin (red).

2. Sorting of IB4-positive DRG neurons

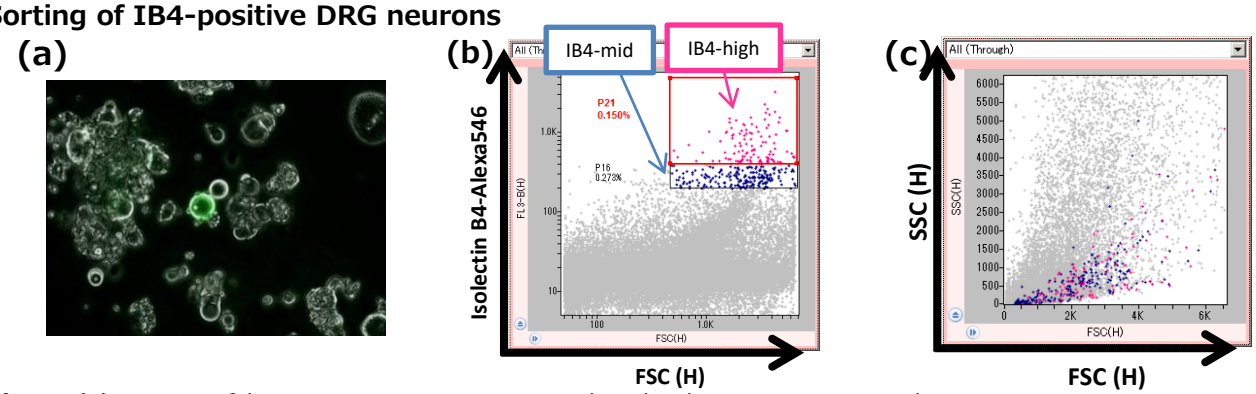

Fig 2. (a) Image of heterogeneous DRGs stained with Alexa546-conjugated anti-IB4 prior to sorting. Note that only the cell highlighted in green at the center of the micrograph was IB4-positive. (b) Anti-IB4-Alexa546 vs. FSC plot of stained DRG cells on On-chip Sort. Red box represents sorting gate for cells with high IB4 expression. (c) SSC-FSC plot of (b).

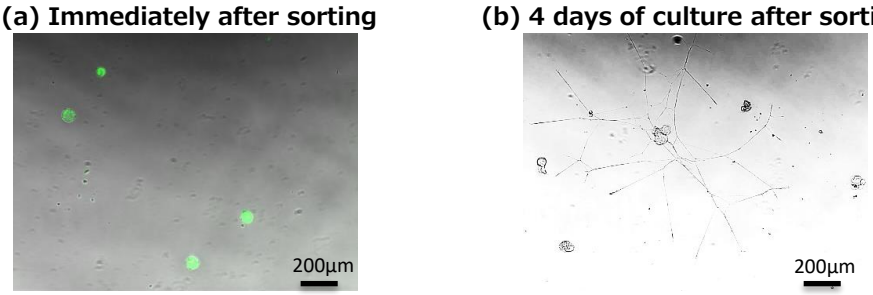

Fig 3. Images of isolated DRG neuron cells with high cell surface IB4 binding protein expression immediately after sorting (a) and four days of culture after sorting (b).

DRG neurons are known to exist in two types: peptidergic neurons and non-peptidergic neurons. There have been reports that IB4 binding protein on cell surface preferentially binds to non-peptidergic neurons[1,2], and thus the two types of neurons can be differentiated by the binding of IB4. The presence of IB4-positive cells in the stained heterogeneous DRGs were confirmed by microscopy (Fig. 2a). After sorting by On-chip Sort, cell populations with high and medium expressions of IB4 (indicated as 'IB4-high' and 'IB4-mid', respectively, on Fig. 2b) were found. The correlation between IB4-binding protein expression and cell size or cell complexity was also investigated (Fig. 2c). However, the results showed no relationship between the two parameters. The "IB4-high" group, representing 0.15% of the overall cells, was isolated for culture for four days. Isolated cells highly expressing IB4 showed neurite elongation after four days of culture (Fig. 3). Therefore, the results suggested that IB4-positive neurons remained undamaged and viable post-sorting by On-chip Sort.

Summary

On-chip Sort was able to isolate pain-related DRG neurons from a heterogeneous cell population by size. On-chip Sort was also capable of isolating cells with high expression of IB4 binding protein on cell surface. In both occasions, culture medium was used as sheath liquid, and isolated cells were confirmed to be undamaged and viable after four or five days of culture post-sorting on On-chip Sort.

References

1) C. L. Stucky, and G. R Lewin. (1999). Isolectin B4-positive and –negative nociceptors are functionally distinct. *The Journal of Neuroscience*. 19(15): 6479-6505.
2) N. J. Fudge, and K. M. Mearow. (2013). Extracellular matrix-associated gene expression in adult sensory neuron populations cultured on a laminin substrate. *BMC Neuroscience*, 14:15.

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