Removal of myelin debris after tissue dissociation optimizes immunomagnetic sorting of cells from postnatal brain




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Introduction

Myelin is a specialized membrane that ensheathes and insulates axons in the peripheral and central nervous system. Myelination in mice and rats begins in the spinal cord around the time of birth and is completed in the brain during the first postnatal month.[1] Myelin formation in humans starts in the spinal cord during the second half of fetal life, peaks during the first year postnatally, and can continue until 20 years of age.[2]

Large quantities of myelin debris are generated when neural tissue is dissociated. Myelin debris in single-cell suspensions has been reported to considerably impair cell isolation and antibody staining.[3,4] Here we present a method of myelin depletion based on magnetic cell sorting (MACS® Technology).

Results

Postnatal (P22) mouse brains were dissociated using the Neural Tissue Dissociation Kit (Papain) and the resulting single-cell suspension analyzed by flow cytometry either before or after treatment with Myelin Removal Beads (Fig. 1). Myelin membrane fragments were extremely abundant prior to depletion with cells representing only 4% of the detected events.

Sucrose solution is occasionally used to deplete myelin from cell samples and so we next directly compared the two depletion methods both in terms of resulting cell viability and efficacy. Myelin Removal Beads produced higher yields of viable cells from the same sized initial population compared to treatment with sucrose solution (Fig. 2).

The efficacy of Myelin Removal Beads was also much higher when compared to sucrose solution with myelin fragments being almost totally depleted (Fig. 3).

We next investigated whether removal of myelin debris prior to MACS Cell Separation would enhance the purity of the isolated target cells (Fig. 4). The purity of cell samples isolated from postnatal mouse brain tissue and positively selected for prominin-1$^+$ expression without prior depletion of myelin fragment was fairly high at 80%. However, removal of myelin fragments before MACS Cell Separation increased the purity of the target cell samples to 95%.

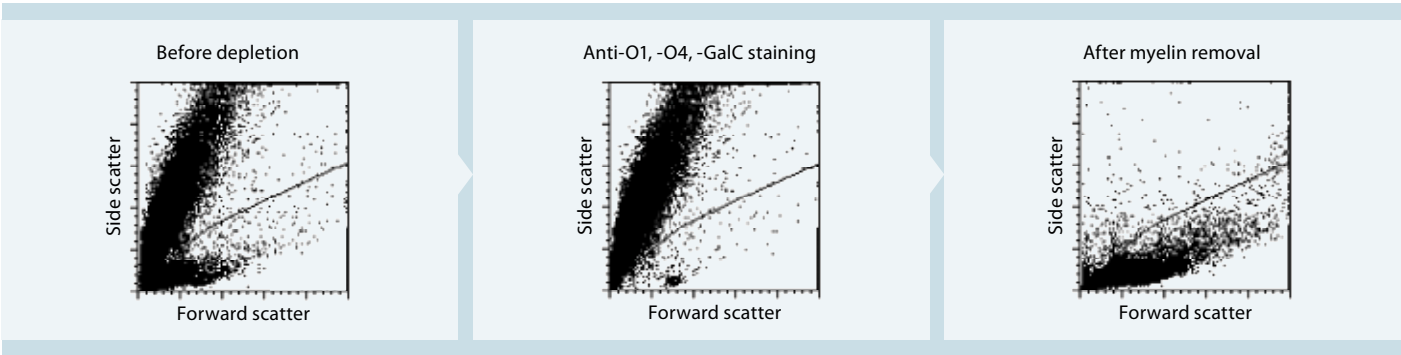

Figure 1. Myelin debris is abundant in single-cell suspensions
Top panel, Single-cell suspensions derived from mouse brain contain abundant myelin fragments. Middle panel, Particles outside the selected region stain with anti-O1, anti-O4 and anti-GalC antibodies that recognize glycolipids contained within myelin membranes. Bottom panel, Myelin Removal Beads used with LD Columns and VarioMACS Separators efficiently remove myelin debris.

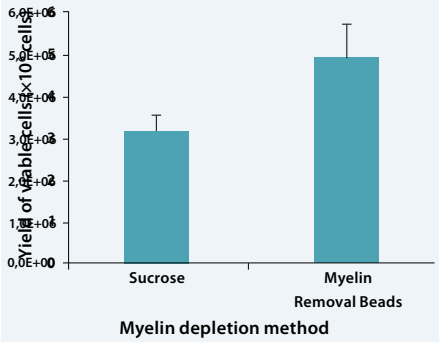

Figure 2. Cell viability following myelin depletion
A higher yield of viable cells is recovered from an initial population of 1×10^7 cells following myelin depletion with Myelin Removal Beads compared to using sucrose solution.

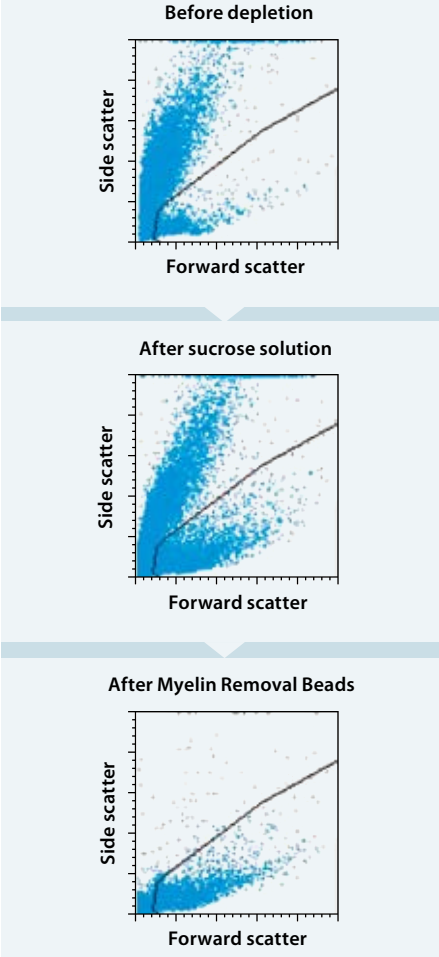

Figure 3. Myelin Removal Beads are more efficient than sucrose solution for removal of myelin debris
Flow cytometry data show the quantity of myelin debris and cells before depletion, after treatment with sucrose solution and after depletion using Myelin Removal Beads. Dead cells were labeled with PI and excluded from the analysis.

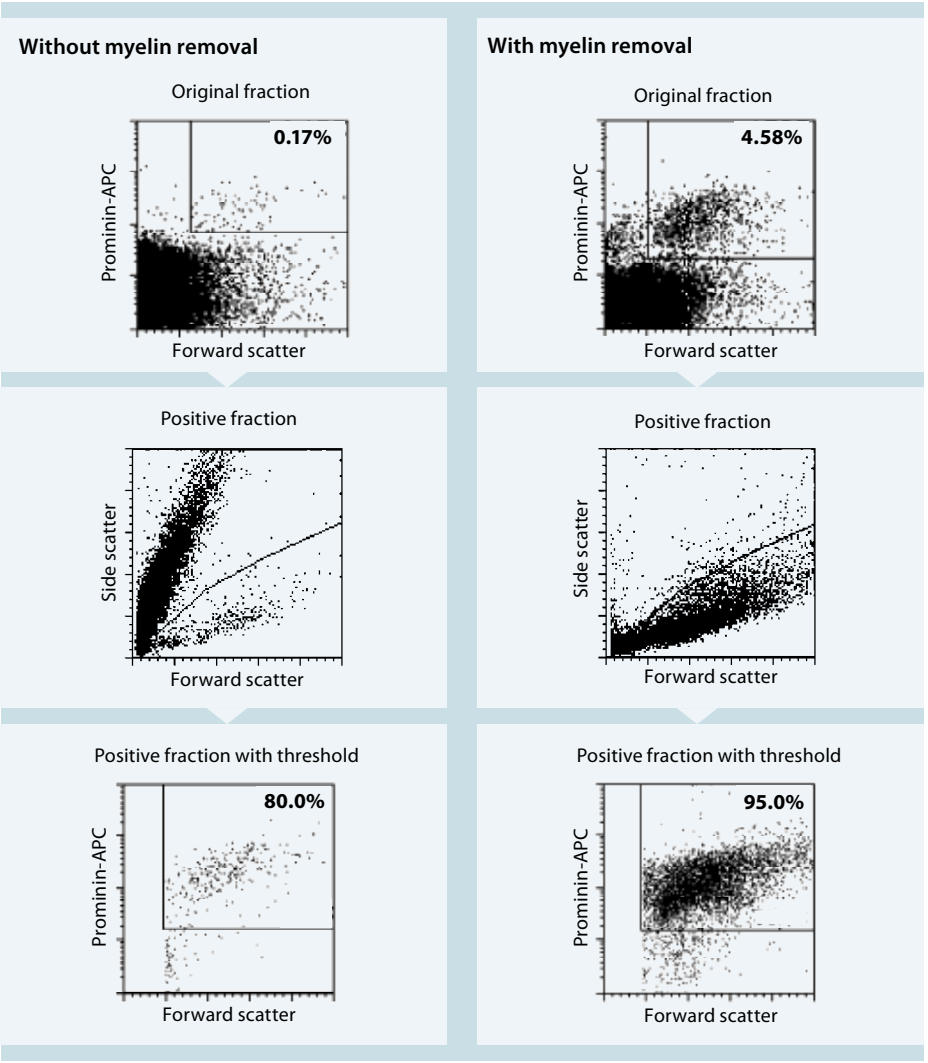

Figure 4. Removal of myelin debris increases the efficiency of MACS Cell Separation
Postnatal (P22) mouse brains were dissociated using the Neural Tissue Dissociation Kit (P) prior to MACS Cell Separation using Anti-Prominin-1 MicroBeads. 1×10^7 cells from the single-cell suspension were then used either directly for cell separation or were submitted to myelin depletion using Myelin Removal Beads prior to cell separation.

Conclusion

- Myelin Removal Beads efficiently deplete myelin debris from cell suspensions
- The recovery of viable cells after Myelin Removal Beads is higher than if sucrose solution is used
- Removal of myelin significantly improves immunostaining and MACS Cell Separation.

References

1 Simons, M. and Trotter, J. (2007) Curr. Opin. Neurobiol. 17: 533–540.
2 Baumann, N. and Pham-Dinh, D. (2001) Physiol. Rev. 81: 871–927.
3 Tham, C.S. *et al.* (2003) Int. J. Dev. Neurosci. 21: 431–443.
4 Pfenninger, C. V. *et al.* (2007) Cancer Res. 67: 5727–5736.

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