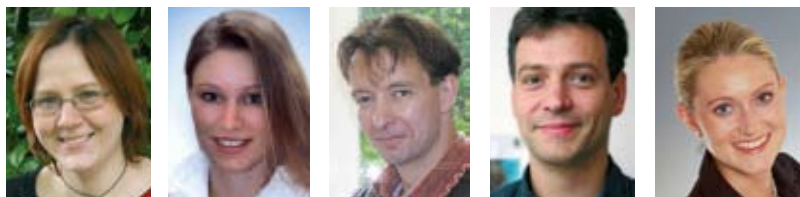


Semi-automated tissue dissociation and preserved epitope integrity optimize immunomagnetic sorting of neural cells



Sandy Reiß, Ina Herzig, Jürgen Schmitz, Andreas Bosio, and Sandra Pennartz
Miltenyi Biotec GmbH, Bergisch Gladbach, Germany

Introduction

Manual mechanical dissociation of tissues leads to fluctuations in the yield of viable cells due to varying pipette sizes and speeds. We have previously shown that using the Neural Tissue Dissociation Kit (NTDK) for enzymatic dissociation of whole mouse brain tissue or specific regions, such as the subventricular zone, results in high yields of viable single cells (see page XXX). In order to increase the reproducibility of enzymatic tissue dissociation and to facilitate sample processing, we tested the NTDK protocol with a semi-automated mechanical dissociation system; the gentleMACS™ Dissociator. This small, benchtop instrument produces a single-cell suspension from tissue placed within a sterile, disposable tube and substitutes for mincing the tissue into small pieces with scapels and Pasteur pipettes.

Destruction of cell surface epitopes can also dramatically influence the performance of

cell separations. It can either decrease the yield of target cells or change the outcome when a separation strategy combining several markers is used. It is important therefore to choose the appropriate protease according to the antigen used for isolation. Papain is often viewed as a mild protease, while trypsin treatment is regarded as harsh and causing detrimental effects to epitope integrity. We show that this common perception does not apply to a number of antigens and, in some cases, the opposite is true.

Results

Whole brain tissue from P1, P8, and P23 mice was dissociated using the Neural Tissue Dissociation Kit (Papain), either manually or in combination with the gentleMACS™ Dissociator, and then cell viability was measured by flow cytometry (Fig. 1). Very similar

yields of viable cells were generated from both dissociation methods. Those obtained from the gentleMACS™ Dissociator showed less variability.

Prominin-1⁺ cells, PSA-NCAM⁺ cells, and microglia were isolated from single-cell suspensions by MACS® Technology following the use of a NTDK in combination with either manual dissociation or the gentleMACS™ Dissociator (Fig. 2). The purity of the cell populations obtained via selection of each of these three antigens was almost identical irrespective of which tissue dissociation method was used.

Flow cytometric analysis following dissociation of P1 brain tissue with either the NTDK (Papain) or the NTDK (Trypsin) demonstrates the effect of different proteases upon subsequent antibody labeling of antigens (Fig. 3 and summarized in Table 1).

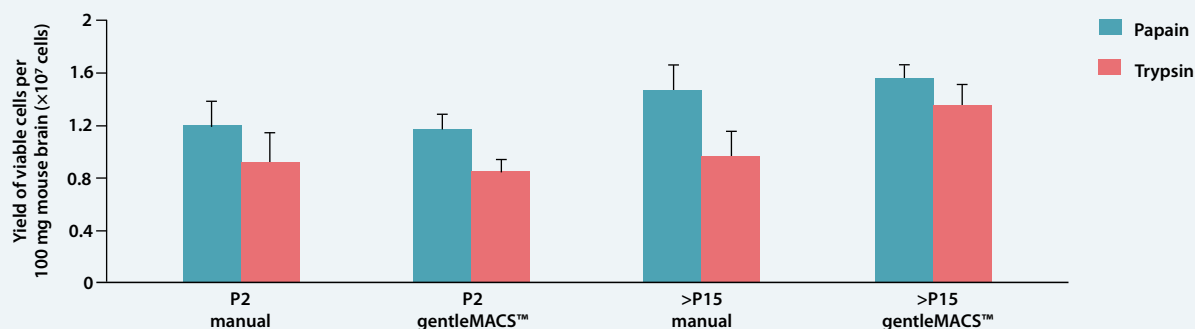


Figure 1. Cell viability: gentleMACS™ Dissociator vs. manual dissociation

Single-cell suspensions generated by the gentleMACS Dissociator contain a similar number of viable cells as those generated by manual dissociation but with less variability in the yield.

Figure 2. MACS Cell Separations: gentleMACS™

Dissociator vs. manual dissociation

Prominin-1⁺ cells were isolated from P22 mouse brain tissue using the Neural Tissue Dissociation Kit (Papain) and Anti-Prominin-1 MicroBeads. The Neural Tissue Dissociation Kit (Trypsin) was used for the isolation of neuronal precursor cells from P1 mouse brain with Anti-PSA-NCAM MicroBeads. Microglia were separated from P14 mouse brain samples using the Neural Tissue Dissociation Kit (Papain) and CD11b (Microglia) MicroBeads.

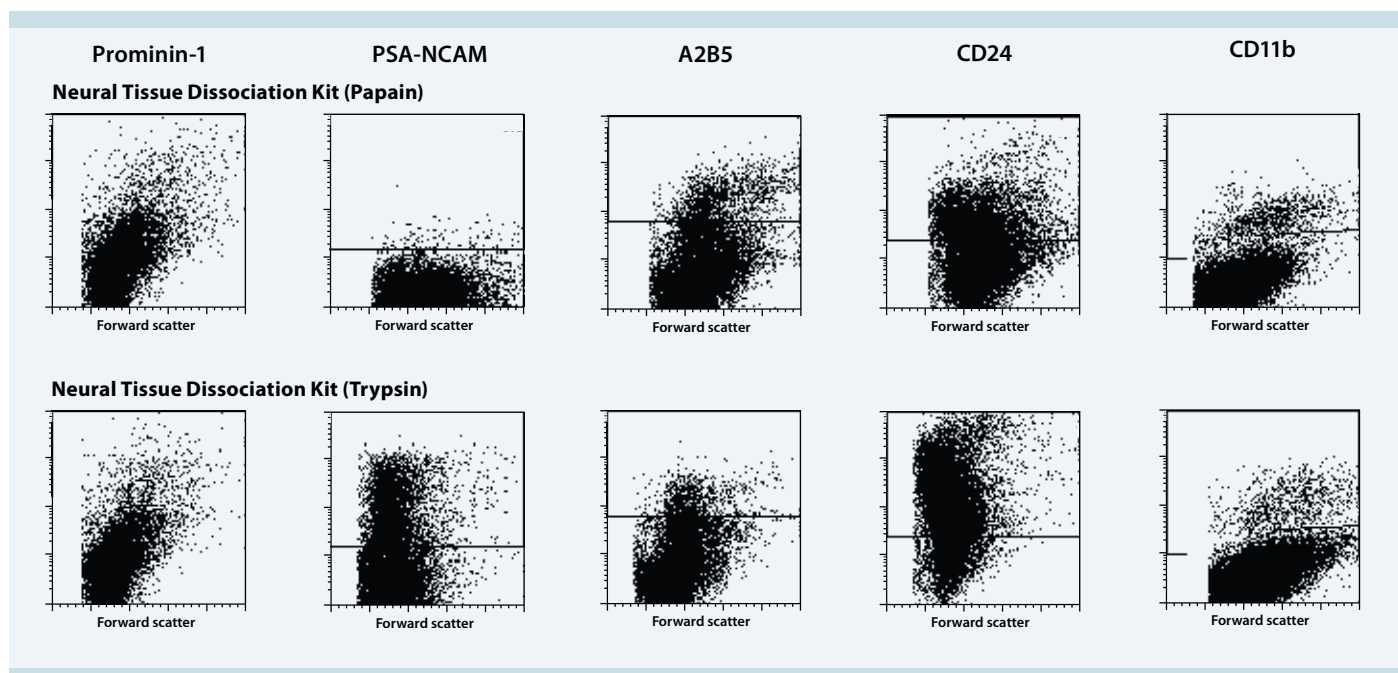
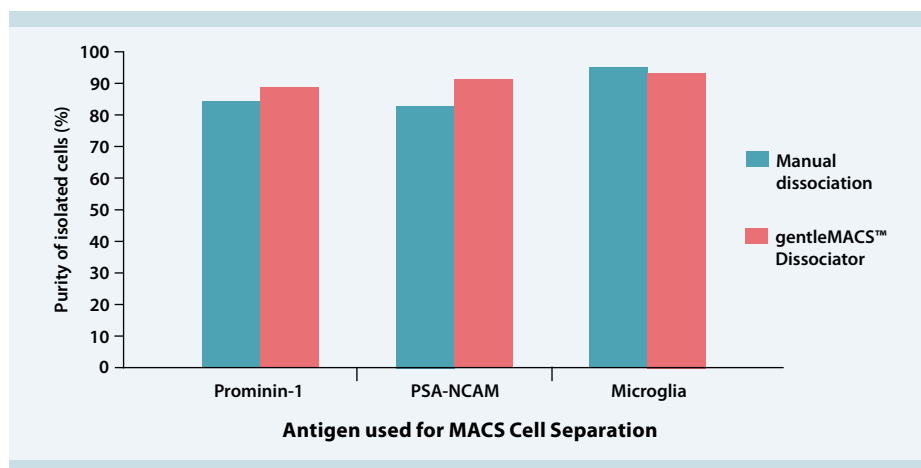


Figure 3. Effect of different proteases on epitope integrity

P1 mouse brain tissue was dissociated with either the Neural Tissue Dissociation Kit (Papain) or the Neural Tissue Dissociation Kit (Trypsin) before cell samples were labeled with antibodies against neural antigens and analyzed by flow cytometry.

Antigen	Trypsin-sensitive?	Papain-sensitive?	Cell type
Prominin-1	Very weakly	No	Neural progenitor cells
PSA-NCAM	No	Yes	Neuronal or oligodendrocyte precursors
A2B5	Weakly	No	Glial precursors
CD24	No	Yes	Neuronal precursors, ependymal cells, erythrocytes
CD11b	No	No	Microglia
CD31	Yes	No	Endothelial cells
CD15 (LeX)	Very weakly	Very weakly	Neural progenitor cells
O4	Yes	Weakly	Immature oligodendrocytes
CD81	Yes	Weakly	Microglia, endothelial cells, glia

Table 1. Summary of neural antigen sensitivities to trypsin and papain

Conclusion

- The gentleMACS™ Dissociator facilitates and standardizes mechanical tissue dissociation
- The Neural Tissue Dissociation Kit (Papain) or the Neural Tissue Dissociation Kit (Trypsin) in combination with the gentleMACS™ Dissociator are effective in generating single-cell suspensions from neural tissues prior to subsequent applications, such as MACS® Cell Separation
- Different antigens exhibit specific sensitivities to either trypsin or papain or both
- The optimal choice of enzyme used for tissue dissociation depends primarily on the epitope of interest