Cell culture

Dissociation with TrypLE Express Enzyme maintains cell health and preserves surface marker expression

Introduction

A critical step in the subculture of adherent cells is the dissociation of cells from the cell culture dish once they approach confluence. Choosing the right dissociation reagent is crucial to maintaining both cell health and relevant phenotypes, including the expression of surface proteins. Trypsin, which acts by breaking down the proteins that facilitate adhesion to the culture dish, has historically been the most commonly used dissociation reagent. However, trypsin comes with several drawbacks. It is an animal-derived enzyme, and therefore poorly defined, with the potential for lot-to-lot variation. Depending on the cell type and duration of exposure, treatment with trypsin can result in the degradation of critical cell surface proteins and glycoproteins [1-3]. As an alternative to trypsin, we offer Gibco™ TrypLE™ Express Enzyme, a gentler, animal origin–free* dissociation reagent. TrypLE Express Enzyme is a recombinant protein that works on the same principle as trypsin, and its higher purity increases specificity and reduces damage to cells that can be caused by other enzymes that may contaminate some trypsin extracts [4].

The study presented here aimed to assess the impact of harvesting cells using trypsin and TrypLE Express Enzyme on viability and surface marker expression of different cell types. Viability of the dissociated cells was assessed by a trypan blue exclusion assay, and the relative difference in expression of cell surface markers was determined by flow cytometric analysis. Cell types used for this study were RAW264.7 macrophages, M1 macrophages derived from THP-1 cells (THP-1-M1 cells), tumor-associated macrophages (TAMs), bone marrow–derived mesenchymal stromal cells (BMMSCs), and induced pluripotent stem cells (iPSCs).

In this study, we show that length of exposure to trypsin had a significant impact on the expression of surface antigens. For cell types whose dissociation time was shorter, we found comparable expression of surface markers upon treatment with trypsin and TrypLE Express Enzyme. However, macrophages, which adhere strongly to the surface, showed reduced expression of surface markers after treatment with trypsin for long periods, as compared to treatment with TrypLE Express Enzyme, indicating the safety of using this reagent for prolonged periods if needed.

* TrypLE Express Enzyme is animal origin–free (AOF) at the primary, secondary, and tertiary levels. It is not produced on AOF-exclusive equipment.

Results

Effect of trypsin and TrypLE Express Enzyme on cell viability

All cells were grown at 37°C in a humidified atmosphere with 5% CO_2 in the recommended media and conditions. Upon reaching confluence, cells were dissociated using either trypsin (0.25% Gibco™ Trypsin-EDTA) or TrypLE Express Enzyme. The results (Figure 1) showed that cell viability was equivalent with the two treatments on different types of macrophages, even though the dissociation time was as long as 50 minutes for RAW264.7 cells and 30 minutes for THP-1-M1 cells and TAMs. Similarly, there was no significant difference between the two dissociation treatments in their effect on cell viability, in BMMSCs and iPSCs.

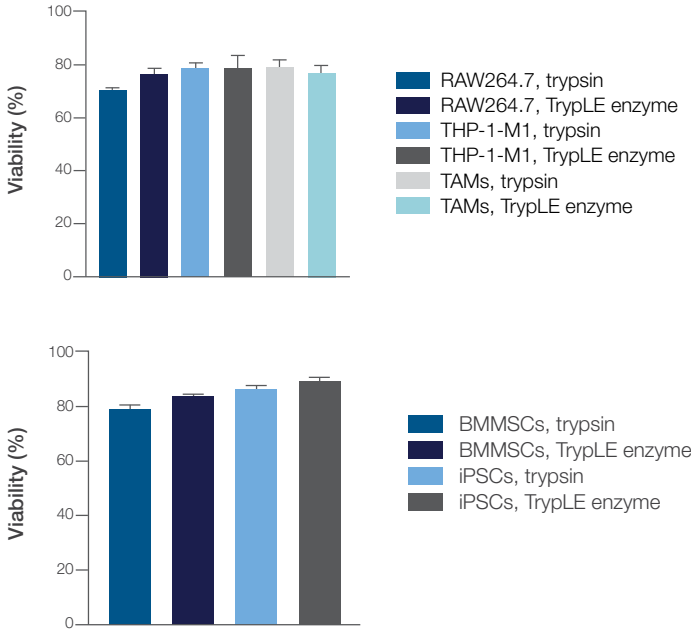

Figure 1. Viability of various cell types dissociated using trypsin and TrypLE Express Enzyme. TAM: tumor-associated macrophage; BMMSC: bone marrow–derived mesenchymal stromal cell; iPSC: induced pluripotent stem cell.

Effect of trypsin and TrypLE Express Enzyme on the expression of cell surface markers

Following viability assessment, we examined the effect of trypsin and TrypLE Express Enzyme on the expression of surface markers via flow cytometry. Trypsin treatment significantly reduced the expression of CD11b and CD44, as compared to TrypLE Express Enzyme, in RAW264.7 cells (Figures 2A, B). Differences in surface marker expression were also observed in THP-1-M1 cells (Figures 2C–F) and TAMs (Figures 2G, H). Our data demonstrate that TrypLE Express Enzyme had a less pronounced effect than trypsin on expression of different cell surface markers.

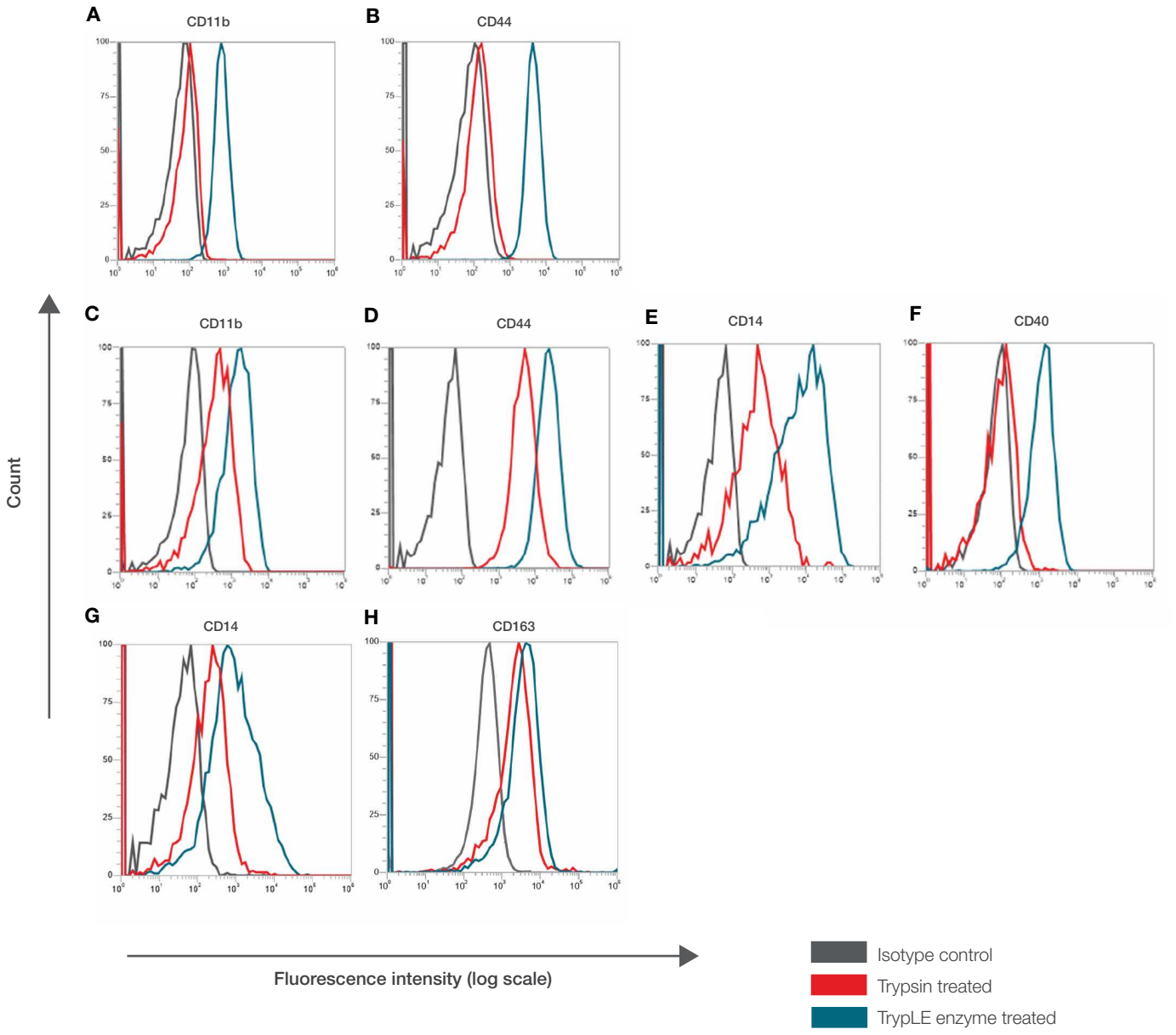

Figure 2. Surface markers on macrophages treated with trypsin and TrypLE Express Enzyme. Shown are markers for **(A, B)** RAW264.7 cells, **(C–F)** THP-1-M1 cells, and **(G, H)** TAMs.

Apart from macrophages, we also studied the effects of trypsin and TrypLE Express Enzyme on sensitive cells like MSCs and iPSCs, whose dissociation times are shorter than those of macrophages. Previous data have shown time-dependent degradation of surface markers for these cell types [4]. In our study, the dissociation time for these cells using both enzymes was approximately 1/10 that of macrophages. As shown in Figure 3, cells treated with trypsin and TrypLE Express Enzyme showed similar cell surface marker expression, a result suggesting that differences between these enzymes may be emphasized by longer exposure times.

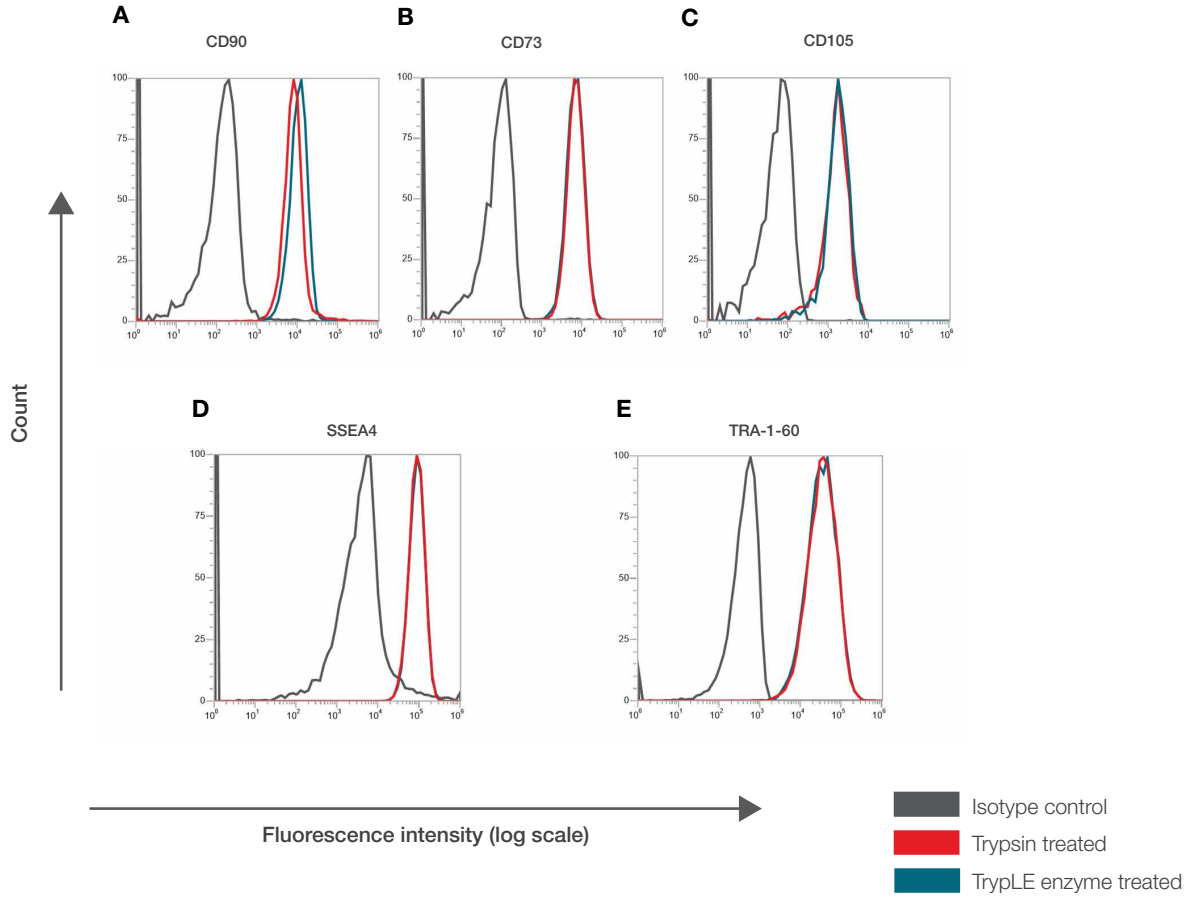

Figure 3. Surface markers on cells treated with trypsin and TrypLE Express Enzyme. Shown are markers for **(A–C)** MSCs and **(D, E)** iPSCs.

Conclusion

To summarize, this study demonstrated that duration of trypsin treatment (determined by the length of time needed to complete dissociation of cells from the culture vessel surface) had a noticeable effect on preservation of surface markers. Longer incubation times significantly reduced the levels of certain surface markers in macrophages, but incubation time had no measurable impact on cells where the dissociation time was considerably shorter. On the other hand, the gentle enzymatic activity of TrypLE Express Enzyme preserves the expression of surface markers regardless of the exposure time without impacting cell viability.

Overall, our study supports the fact that it is important to select an appropriate detachment method based on the experimental design. As an effective and gentler reagent, TrypLE Express Enzyme might be an ideal choice for extended exposure times.

Ordering information

Description	Cat. No.
TrypLE Express Enzyme (1X), phenol red	12605010
Trypsin-EDTA (0.25%), phenol red	25200056
Trypan Blue Solution, 0.4%	15250061
CD11b Monoclonal Antibody, FITC, eBioscience	11-0112-82
CD163 Monoclonal Antibody, APC, eBioscience	17-1639-42
CD206 Monoclonal Antibody, APC, eBioscience	17-2069-42
CD14 Monoclonal Antibody, PE, eBioscience	12-0149-42
CD40 Monoclonal Antibody, FITC, eBioscience	11-0409-42
CD73 Monoclonal Antibody, FITC, eBioscience	11-0739-42
CD105 Monoclonal Antibody, eFluor 450, eBioscience	48-1057-42
CD44 Monoclonal Antibody, PE, eBioscience	12-0441-82
SSEA4 Monoclonal Antibody, PE, eBioscience	12-8843-42
Rat IgG2b Kappa Isotype Control (eB149/10H5), FITC, eBioscience	11-4031-82
Rat IgG2b Kappa Isotype Control (eB149/10H5), PE, eBioscience	12-4031-82
Mouse IgG1 Kappa Isotype Control (P3.6.2.8.1), APC, eBioscience	17-4714-82
Mouse IgG1 Kappa Isotype Control (P3.6.2.8.1), PE, eBioscience	12-4714-82
Mouse IgG1 Kappa Isotype Control (P3.6.2.8.1), FITC, eBioscience	11-4714-81
Mouse IgG1 Kappa Isotype Control (P3.6.2.8.1), eFluor 450, eBioscience	48-4714-82
Mouse IgG3 Isotype Control (B10), PE, eBioscience	12-8843-42
TRA-1-60 Alexa Fluor 488 Conjugate Kit for Live Cell Imaging	A25618

References

1. Liu Q, Zhao R, Huang C et al. (2018) Effects of enzymatic digestion, cell culture and preservation conditions on surface CD62L expression of primary murine CD3$^+$CD4$^+$ T cells. *Biomed Res* 29:2153 –2159.
2. Yan J, Xie C, Zhu J et al. (2021) Effect of trypsin concentration on living SMCC-7721 cells studied by atomic force microscopy. *J Microsc* 284:203–213.
3. Lillico DME, Pemberton JG, Stafford JL (2016) Trypsin differentially modulates the surface expression and function of channel catfish leukocyte immune-type receptors. *Dev Comp Immunol* 65:231–244.
4. Tsuji K, Ojima M, Otabe K et al. (2017) Effects of different cell-detaching methods on the viability and cell surface antigen expression of synovial mesenchymal stem cells. *Cell Transplant* 26:1089–1102.

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