Advantages of UpCell surface over trypsin for preserving cell viability and expression of cell surface antigens

Description

In cell culture, the process of removing cells from a culture substrate, also known as dissociation, is most often accomplished by treatment with a proteolytic enzyme like trypsin. Treatment with trypsin, however, can impact the expression of proteins on the cell surface and may compromise cell health [1]. To avoid such damage and to improve the quality of harvested cells, dissociation via trypsin can be eliminated by using Thermo Scientific™ Nunc™ Dishes with UpCell™ Surface. The UpCell surface, which transitions from hydrophobic to hydrophilic as temperature drops from 37°C to below 32°C, enables the harvest of adherent cells with high viability and intact surface proteins.

In this study, we examined the effect of dissociation using 0.25% trypsin-EDTA and the UpCell surface on the viability and expression of cell surface antigens in cultured mesenchymal stromal cells (MSCs), HT-29 colorectal cancer cells, and RAW 264.7 macrophages.

Materials and methods

Cell culture

All cells types were cultured on Thermo Scientific™ Nunc™ EasYDish™ Dishes with Nunclon Delta™ Surface (Cat. No. 150462) or Nunc™ Dishes with UpCell™ Surface (Cat. No. 174903). Gibco™ StemPro™ Bone Marrow MSCs (Cat. No. A15652) were cultured in Gibco™ MesenPRO RS™ Medium (Cat. No. 12746012) with the included growth supplement. HT-29 cells were grown in Gibco™ McCoy's 5A Medium (Cat. No. 16600082) with 10% Gibco™ Fetal Bovine Serum (Cat. No. 26140079) and 1% Gibco™ Penicillin-Streptomycin (Cat. No. 15140122), as recommended by ATCC. RAW 264.7 cells were grown in Gibco™ DMEM (Cat. No. 41965039) with 10% Fetal Bovine Serum and 1% Penicillin-Streptomycin, as recommended by ATCC.

Harvest of cells from the Nunclon Delta surface using trypsinization

Non-adherent cells were removed by washing the culture dishes with Gibco™ DPBS, no calcium, no magnesium (Cat. No. 14190144). Then, 2 mL of Gibco™ Trypsin-EDTA (0.25%, Cat. No. 25200056) was added to the dishes followed by incubation at 37°C. MesenPRO RS Medium, McCoy's 5A Medium, or DMEM (5 mL) was added to the appropriate dishes to neutralize trypsin. Detachment time was noted, cells were harvested, and cell viability and density were determined using the Invitrogen™ Countess™ II Automated Cell Counter (Cat. No. AMQAX1000).

Harvest of cells from the UpCell surface using temperature reduction

Medium was aspirated from the dishes, which were then washed once with DPBS, no calcium, no magnesium. MesenPRO RS Medium, McCoy's 5A Medium, or DMEM (3 mL) was added to the appropriate dishes. Cultures were incubated at approximately 4°C in a refrigerator, and time taken for cell detachment was noted. Cells were then collected, and cell viability and density were determined using the Countess II Automated Cell Counter.

Flow cytometry analysis

Cells harvested under different conditions were washed and resuspended in 1 mL of 1X Gibco™ PBS (Cat. No. 10010023). Primary antibodies and corresponding isotype control antibodies were added at the recommended concentrations to the cells, followed by incubation at 4°C for 30 min in dark. Invitrogen™ eBioscience™ eFluor™ 450 Fixable Viability Dye (Cat. No. 65-0863-14) was added at 1:1,000 dilution to the cell suspension as well. Cells were then washed using 1 mL 1X PBS and resuspended in 1 mL flow cytometry staining buffer followed by data acquisition.

Results

Morphology of MSCs on the Nunclon Delta and UpCell surfaces

MSCs grown on the Nunclon Delta and UpCell surfaces showed no significant differences in cell attachment, proliferation, or morphology (Figure 1).

Cell viability of dissociated MSCs

Trypsin dissociated MSCs from the Nunclon Delta surface within 5–6 min at 37°C, while temperature shift (to 4°C) required 10–12 min to obtain the maximum cell dissociation from the UpCell surface (Figure 2A). There was no significant difference in cell viability between the two dissociation methods (Figure 2B).

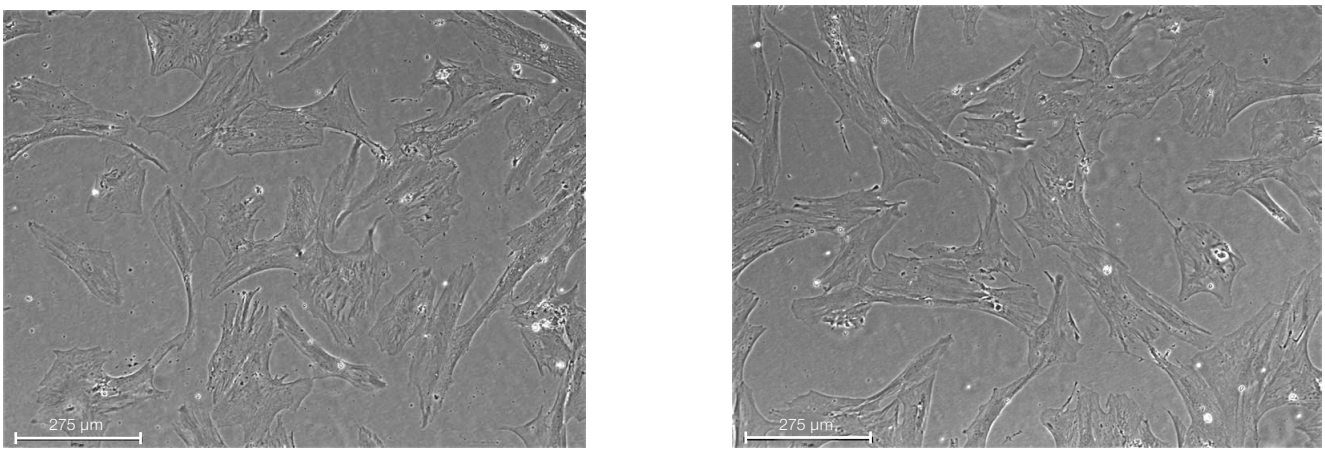


Figure 1. Phase-contrast images of MSCs grown on Nunclon Delta and UpCell surfaces. Images were captured on the Invitrogen™ EVOS™ M7000 Imaging System at 10x magnification (scale bar: 275 µm).

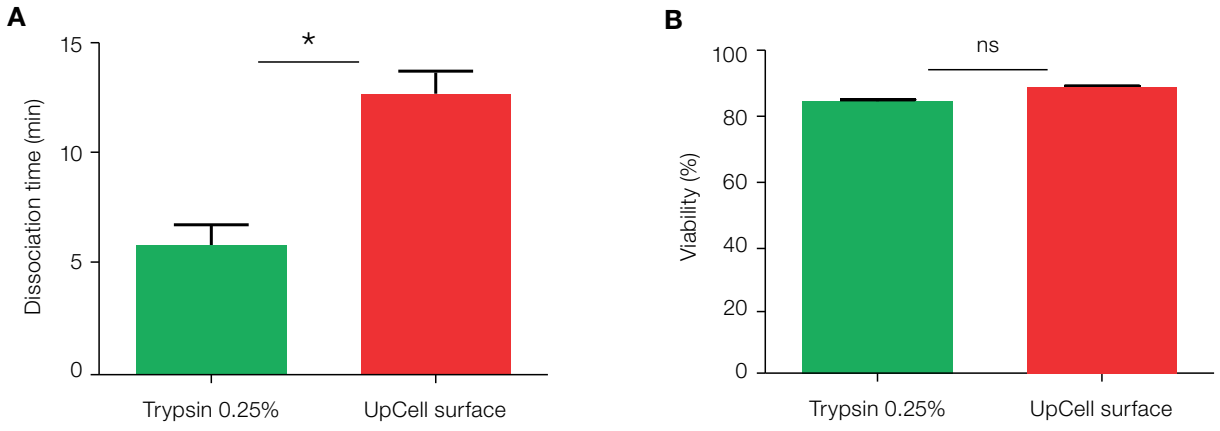


Figure 2. Despite longer dissociation time, the UpCell surface does not impact cell viability. MSCs were treated with trypsin on the Nunclon Delta surface or dissociated by temperature shift on the UpCell surface until cells were completely detached. **(A)** Dissociation time was noted, and **(B)** cell viability was measured using the Countess II Automated Cell Counter. Individual experiments were done in duplicate, and data are represented as mean ± SEM. ns: not significant, *: $P < 0.05$ (two-tailed unpaired *t*-test).

Effect of dissociation on expression of CD44, CD105, and CD13 in MSCs

MSCs are adherent, fibroblast-like cells, and enzymatic digestion is usually required for the preparation of cell suspensions. The detachment and dissociation of MSCs using harsh dissociation reagents can alter cell surface antigen expression profiles, multipotency, and therefore efficacy of MSC transplantation [2,3]. We examined three cell surface antigens expressed on MSCs: CD44, CD105, and CD13. Analyses based on the amino acid sequence of each protein predicted that all three have multiple trypsin recognition sequences, which may lead to cleavage of the proteins [4]. Thus, we wanted to determine if the use of the UpCell surface preserved the expression of these surface markers. Following dissociation, MSCs were stained with Invitrogen™ eBioscience™ monoclonal antibodies against CD44 (Cat. No. 12-0441-82), CD105 (Cat. No. 17-1057-42), and CD13 (Cat. No. 11-0138-42), and expression levels were measured using the Attune NxT Flow Cytometer.

Post-acquisition analysis indicated that the expression of CD44 was reduced by cell dissociation using trypsin treatment, compared to the UpCell surface (Figure 3, left panels). The UpCell surface had a milder effect on CD105 antigenicity relative to trypsin (Figure 3, middle panels). We did not observe any reduction in CD13 expression by trypsin treatment compared to the use of the UpCell surface (Figure 3, right panels). The lesser impact of trypsin on CD105 and CD13 relative to CD44 may be due to posttranslational modifications or the three-dimensional conformation of each antigen. These differences suggest that care should be taken when selecting a dissociation reagent if cell surface antigens are crucial for downstream assays. Taken together, our results indicate that the UpCell surface has milder effects on cultured MSCs, preserving cell surface antigens and maintaining high viability compared to trypsin-mediated dissociation.

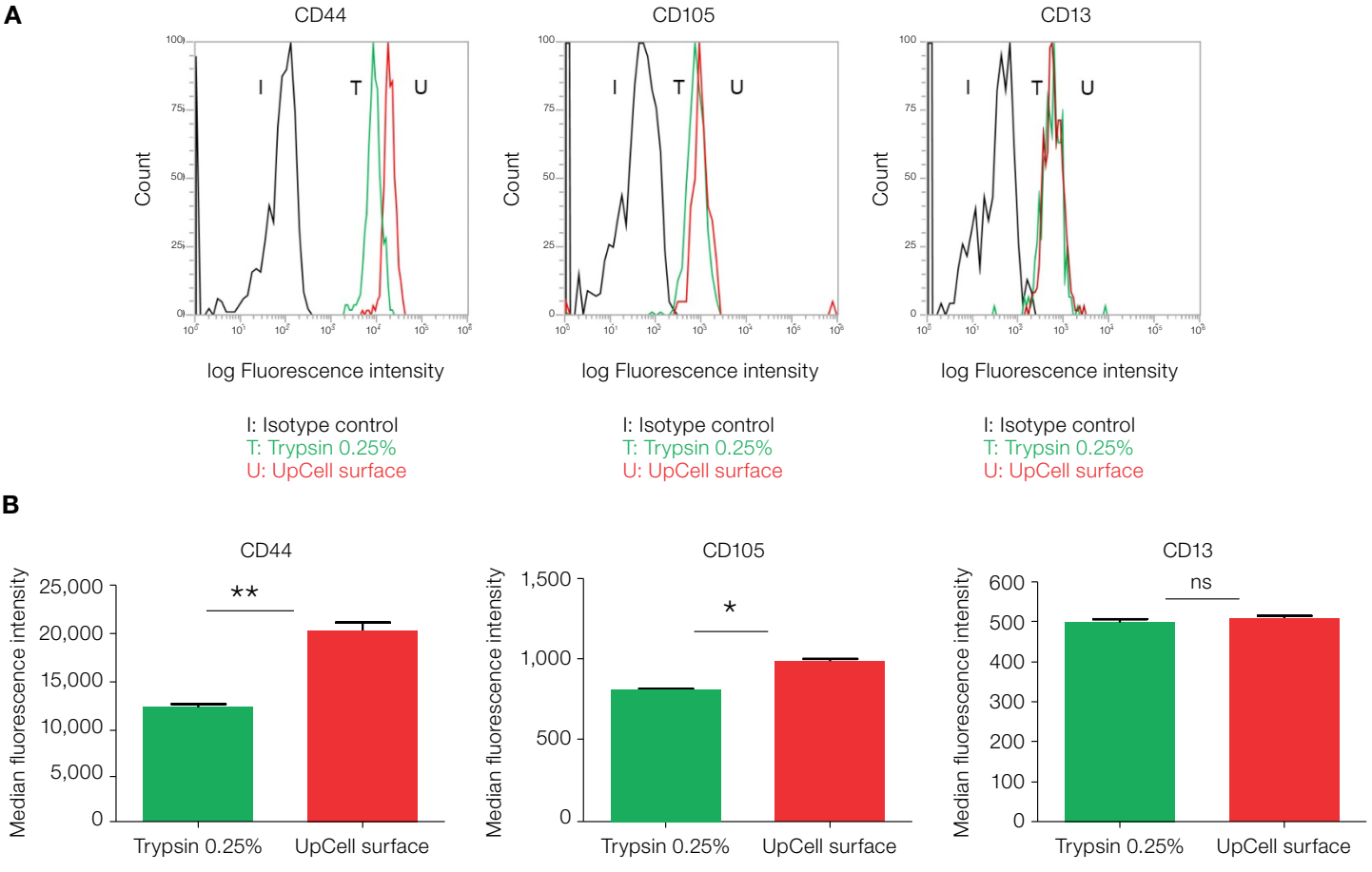


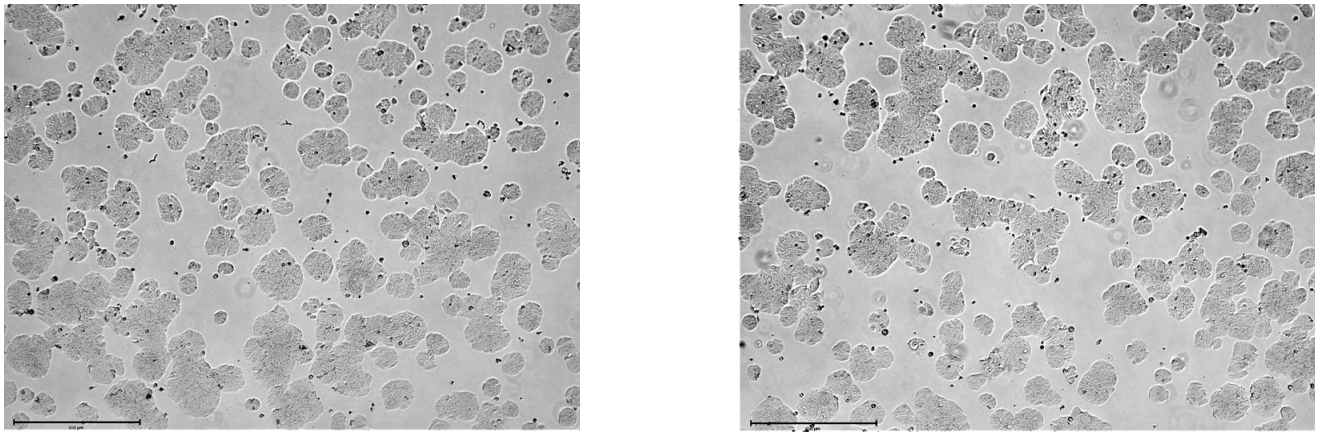
Figure 3. Measurement of CD44, CD105, and CD13 fluorescence intensity in MSCs after antibody staining. (A) Representative flow cytometry histograms showing expression of CD44, CD105, and CD13 following dissociation using 0.25% trypsin or the UpCell surface. **(B)** Median fluorescence intensities of surface antigens are demonstrated as bar graphs. Individual experiments were done in duplicate, and data are represented as mean ± SEM. ns: not significant, **: $P < 0.005$, *: $P < 0.05$ (two-tailed unpaired *t*-test).

Morphology of HT-29 cells on Nunclon Delta and UpCell surfaces

HT-29 cells were grown on the Nunclon Delta and UpCell surfaces. As was the case with MSCs, the UpCell surface showed similar cell attachment, proliferation, and morphology as compared to the Nunclon Delta surface (Figure 4).

Cell viability of dissociated HT-29 cells

Trypsin treatment (at 37°C) or temperature shift (to 4°C) completely dissociated HT-29 cells from each surface in 5 and 7 minutes, respectively (Figure 5A). We did not observe any difference in cell viability between the two dissociation methods (Figure 5B).



Nunclon Delta surface **UpCell surface**

Figure 4. Brightfield images of HT-29 cells grown on Nunclon Delta and UpCell surfaces. Images were captured on the EVOS M7000 Imaging System at 10x magnification (scale bar: 275 µm).

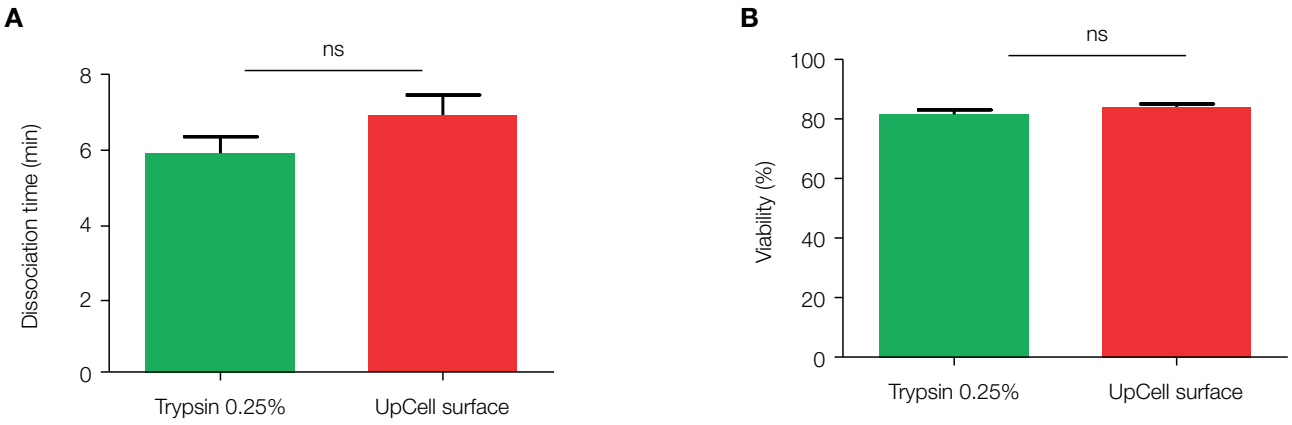


Figure 5. Despite a slight difference in dissociation time, the UpCell surface does not impact cell viability. HT-29 cells were treated with trypsin on the Nunclon Delta surface or dissociated by temperature shift on the UpCell surface until cells were completely detached. **(A)** Dissociation time was noted, and **(B)** cell viability was measured using the Countess II Automated Cell Counter. Error bar represents SEM (2 independent replicates). ns: not significant.

Effect of dissociation on expression of CD44 in HT-29 cells

To confirm the impact of trypsin treatment on extracellular markers, cell surface expression of CD44 was assessed in HT-29 cells after dissociation using trypsin or the UpCell surface. Following dissociation, HT-29 cells were stained with eBioscience CD44 Monoclonal Antibody (Cat. No. 12-0441-82), and expression levels were measured via flow cytometry. As depicted in Figure 6, trypsin treatment significantly reduced the surface expression of CD44 on HT-29 cells, despite the relatively brief exposure to trypsin.

Morphology of RAW 264.7 cells on Nunclon Delta and UpCell surfaces

RAW 264.7 cells were plated on Nunclon Delta and UpCell surfaces and allowed to expand until the cells were approximately 80% confluent. No significant differences were observed when comparing cell attachment, growth, or morphology on the two surfaces (Figure 7).

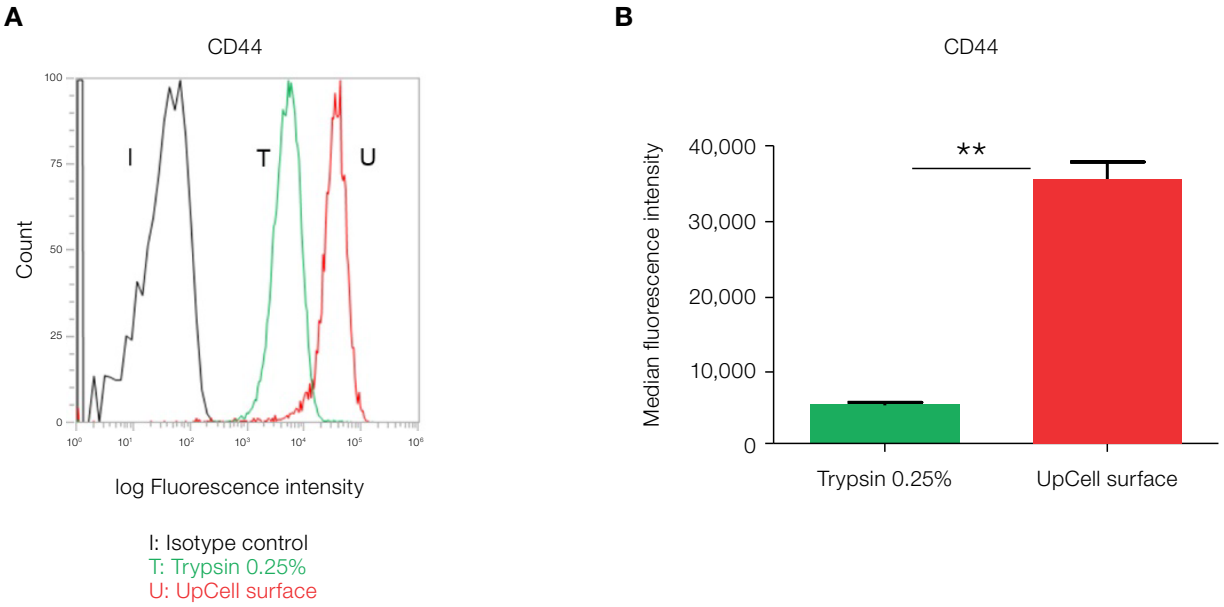
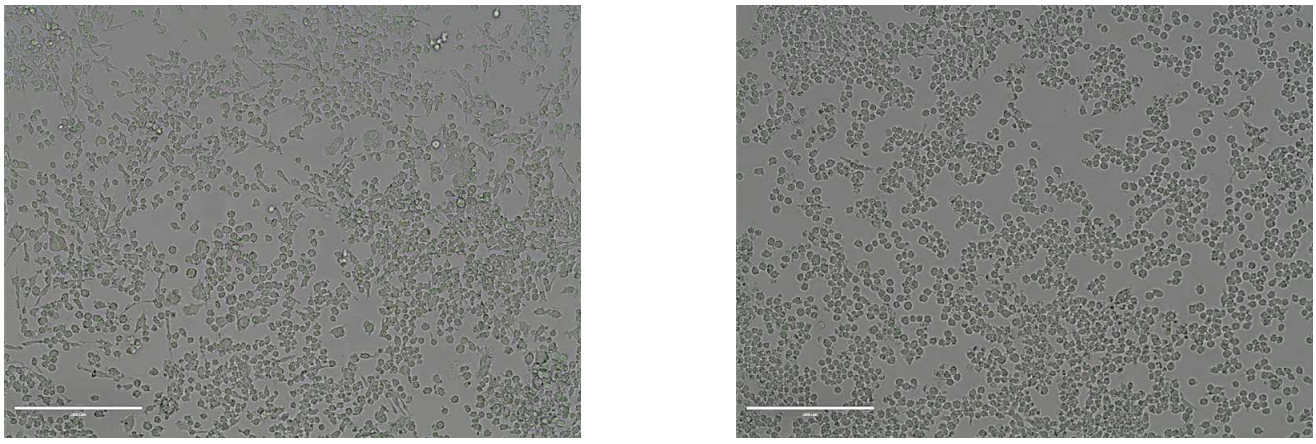


Figure 6. Measurement of CD44 fluorescence intensity in HT-29 cells after antibody staining. (A) Representative flow cytometry histograms showing expression of CD44 following dissociation using 0.25% trypsin or the UpCell surface. **(B)** Median fluorescence intensity of CD44 surface antigen is demonstrated as a bar graph. Individual experiments were done in duplicate, and data are represented as mean ± SEM. **: $P < 0.005$ (two-tailed unpaired *t*-test).



Nunclon Delta surface **UpCell surface**

Figure 7. Brightfield images of RAW 264.7 cells grown on Nunclon Delta and UpCell surfaces. Images were captured on the Invitrogen™ EVOS™ XL Core Imaging System at 10x magnification (scale bar: 200 µm).

Cell viability of dissociated RAW 264.7 cells

To fully dissociate RAW 264.7 cells, Nunclon Delta plates required treatment with trypsin for approximately 70 min at 37°C. Dissociation of these cultures from the UpCell surface required only 15 min at 4°C (Figure 8A). Viability of cells harvested from the UpCell surface was found to be approximately 15% higher than those dissociated with trypsin (Figure 8B).

Effect of dissociation on expression of CD44 and CD11b in RAW 264.7 cells

RAW 264.7 are mouse macrophages and express high levels of CD44 and CD11b. To confirm the impact of trypsin treatment on CD44 and CD11b, cell surface expression of both proteins was assessed in RAW 264.7 cells after dissociation using trypsin or the UpCell surface. Following dissociation, cells were stained with Invitrogen™ eBioscience™ antibodies against CD44 (Cat. No. 12-0441-82) and CD11b (Cat. No. 11-0112-82), and expression levels were measured via flow cytometry. Our analysis indicated that the expression of both CD44 and CD11b was reduced by >80% with trypsin treatment relative to the UpCell surface (Figure 9). Taken together, our results indicate that dissociation via the UpCell surface offers significant improvements for firmly adherent cell types like RAW 264.7. Compared to trypsin, the UpCell surface drastically reduced dissociation time, maintained higher viability, and preserved critical cell surface antigens.

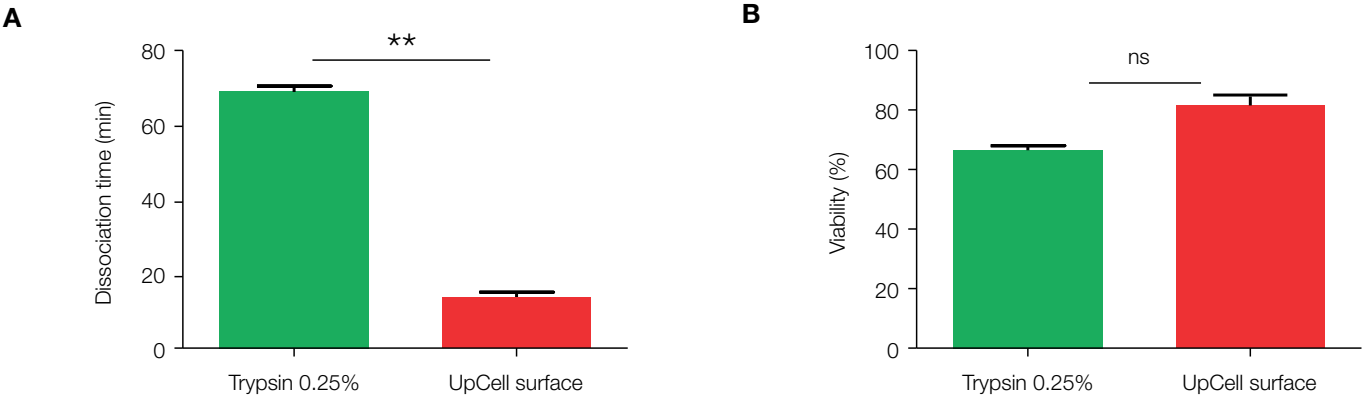


Figure 8. Faster dissociation of RAW 264.7 cells from the UpCell surface. RAW 264.7 cells were treated with trypsin on the Nunclon Delta surface or dissociated at 4°C on the UpCell surface until cells were completely detached. **(A)** Dissociation time was noted, and **(B)** cell viability was measured using the Countess II Automated Cell Counter. Error bar represents SEM (2 independent replicates). ns: not significant, **: $P < 0.005$ (two-tailed unpaired *t*-test).

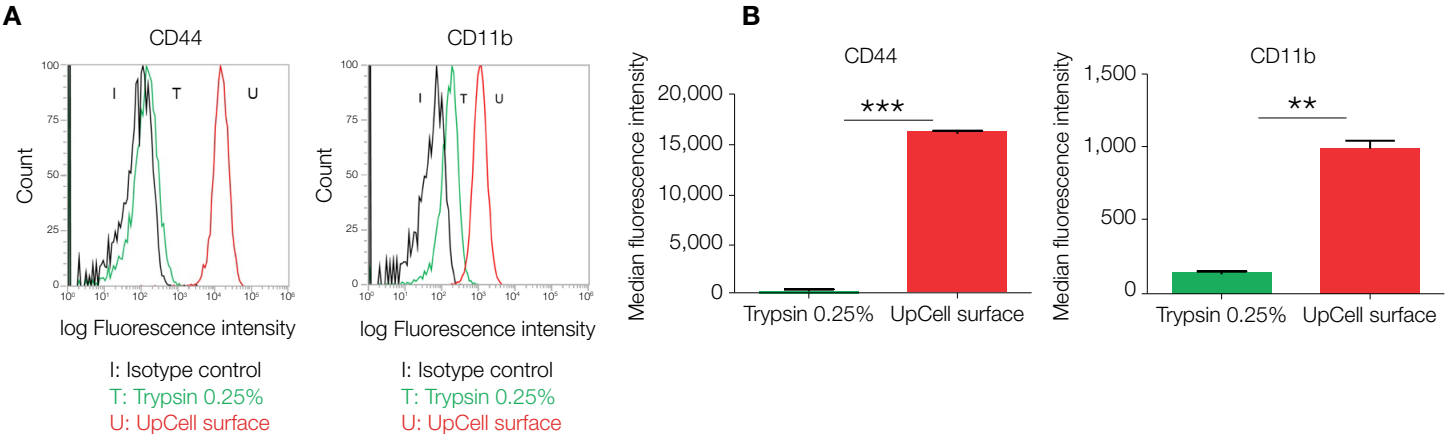


Figure 9. Measurement of CD44 and CD11b fluorescence intensity in RAW 264.7 cells after antibody staining. (A) Representative flow cytometry histograms showing expression of CD44 and CD11b following dissociation using 0.25% trypsin or the UpCell surface. **(B)** Median fluorescence intensities of surface antigens are demonstrated as bar graphs. Individual experiments were done in duplicate, and data are represented as mean ± SEM.
**: $P < 0.005$, ***: $P < 0.0001$ (two-tailed unpaired *t*-test).

Conclusion

These results indicate that the UpCell surface preserves the antigenicity of some surface markers, including CD44 and CD11b, better than trypsin. Flow cytometric analyses of MSCs, tumor cells, and macrophages indicated that surface antigens can be significantly influenced by enzymatic digestion conventionally used for cell dissociation. Among the antigens tested, expression of CD44 and CD11b was reduced by trypsin, CD105 showed only a slight difference between methods, and CD13 showed no observable change. These results suggest that while not all antigens are impacted by trypsin, care should be taken when choosing an enzymatic dissociation reagent. In addition, RAW 264.7 macrophage cells, which adhere firmly to tissue culture–treated surfaces, dissociated quickly from the UpCell surface, suggesting this surface is an especially effective option for sticky cell lines.

Use of the UpCell surface for dissociation of adherent cell cultures can help avoid the proteolytic effect of trypsin, preserve the structural integrity of membrane surface proteins, and maintain good cell viability for downstream assays.

References

1. Huang HL, Hsing HW, Lai TC et al. (2010) Trypsin-induced proteome alteration during cell subculture in mammalian cells. *J Biomed Sci* 17(1):36.
2. 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(6):1089–1102.
3. Chaudhry MA (2008) Induction of gene expression alterations by culture medium from trypsinized cells. *J Biol Sci* 8(1):81–87.
4. Wilkins MR, Gasteiger E, Bairoch A et al. (1999) Protein identification and analysis tools in the ExPASy server. *Methods Mol Biol* 112:531–552.

Ordering information

Product	Description	Cat. No.
Nunc Dishes with UpCell Surface	35 mm, UpCell dish	174904
	60 mm, UpCell dish	174903
	100 mm, UpCell dish	174902
	60 mm, UpCell dish with grid	174906
	100 mm, UpCell dish with grid	174905
Nunc Multidishes with UpCell Surface	6-well, UpCell multidish	174901
	12-well, UpCell multidish	174900
	24-well, UpCell multidish	174899
	48-well, UpCell multidish	174898
Nunc UpCell Microplates	96-well, UpCell microplate, flat bottom	174897

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