thermo scientific

Cell harvesting by temperature reduction

Nunc UpCell Surface

ThermoFisher SCIENTIFIC

Features and benefits

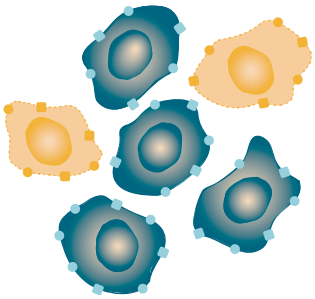
Preserving cell surface proteins

The Thermo Scientific™ Nunc™ UpCell™ Surface enables harvesting of cells with high viability and intact surface proteins for culture passaging, single-cell analyses, and cell transplantation research.

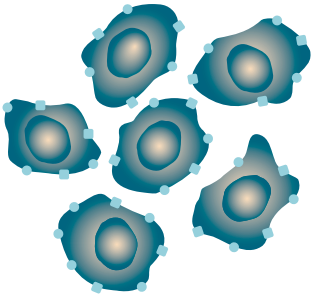
- No trypsin—preserves cell surface proteins
- No scraping—gets high cell viability
- Minimal hands-on time
- Quick, clean, and simple—just reduce the temperature

Cell harvesting using enzymatic digestion, such as trypsinization, results in degradation of cell surface proteins (Application Note 1). These proteins are important for the interactions between the cell and the environment. For example, cell surface proteins are involved in the cell's response to the extracellular matrix, to other cells, and to growth factors and other soluble mediators. Some cell surface proteins are involved in the ion homeostasis of the cell, whereas other cell surface proteins are used as antigens or markers in cell analysis and enrichment procedures.

Cells harvested
by trypsinization



Cells har vested from UpCell Surface
by temperature reduction



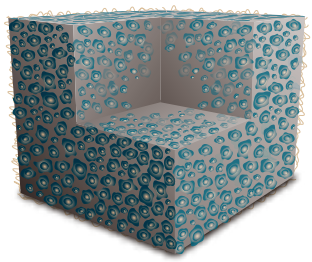
Creating 3D tissue models

The Nunc UpCell Surface enables harvesting of cell sheets and creation of tissue models held together by normal cell junctions and extracellular matrix deposited by the cells.

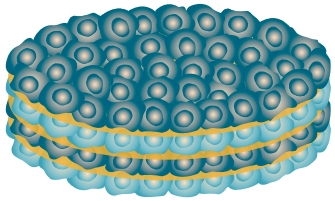
- No scaffold—make three-dimensional (3D) tissue models without exogenous material
- No uneven cell distribution—control the spatial distribution of cells in 3D
- Endless possibilities for mixing cell types and creating 3D co-cultures
- Just harvest and stack cell sheets

In tissue engineering, 3D tissue models or transplants are typically prepared by seeding a cell suspension on a pre-fabricated scaffold. Scaffold materials are not produced by the cells to reside in the engineered tissue, and are most often materials foreign to the body or from another species (xenogeneic), such as polylactic acid (PLA), polyglycolic acid (PGA), alginate, gelatin, and collagen. Problems often encountered using scaffolds for tissue engineering include uneven cell distribution and difficulties in controlling the spacial distribution of different cell types. After transplantation, there can be host inflammatory reactions and fibrous tissue formation due to the exogenous scaffold material.

Tissue engineered
using scaffold

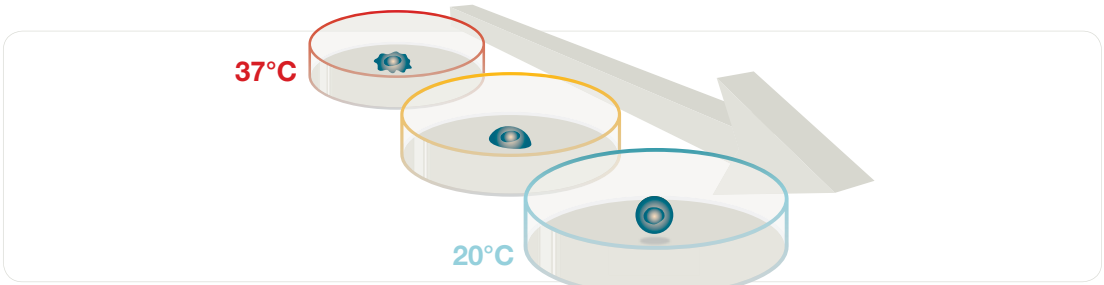


Tissue engineered
using UpCell Surface



Temperature-responsive cell culture surface

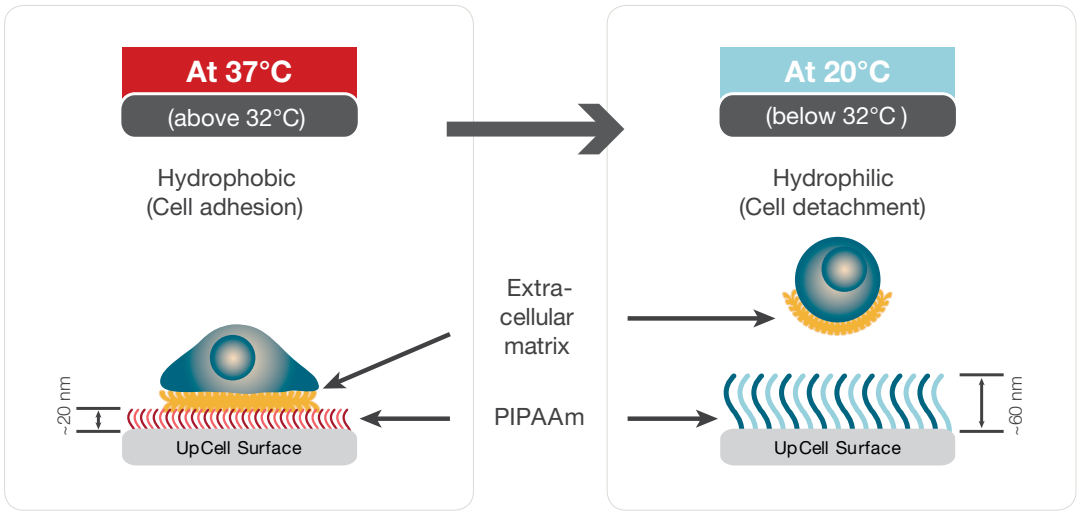
The Nunc UpCell Surface is designed to respond to changes in temperature. It releases adherent cells when there is a reduction of the temperature of the cell culture. Products with Nunc UpCell Surface include Thermo Scientific™ Nunc™ MicroWell™ plates, multidishes, and dishes.



The covalently immobilized polymer poly(N-isopropylacrylamide), or PIPAAm, forms an even and thin layer on the cultureware. The PIPAAm layer is slightly hydrophobic at 37°C, allowing cells to attach and grow. When the temperature of the culture is reduced to below 32°C, the PIPAAm layer becomes very hydrophilic, binds water, and swells, resulting in the release of adherent cells.

Extracellular matrix is harvested with the cells

Depending on the degree of confluence of the culture, and the harvesting technique, single cells or cell sheets can be harvested from the Nunc UpCell Surface. Because the extracellular matrix under the cultured cells is harvested with the cells, cell sheets are naturally adhesive to other cell sheets and to cell surfaces in the body.



Working with single cells

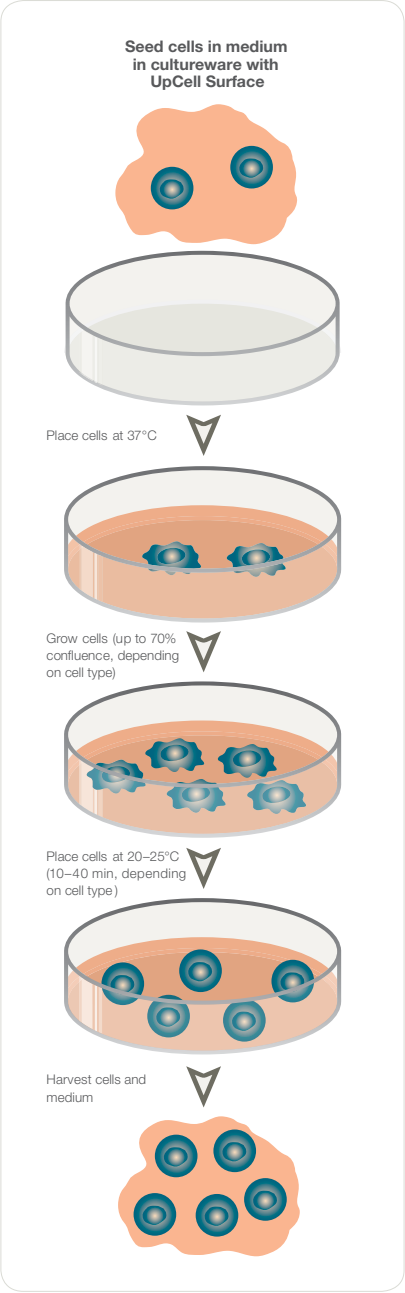
Single-cell suspensions harvested from cultureware with Nunc UpCell Surface can be utilized in the following ways:

- Analyzed *in vitro*, for example, by flow cytometry
- Manipulated to purify certain cell types
- Re-seeded in cultureware with Nunc UpCell Surface or traditional cell cultureware as part of a passaging procedure
- Used in cell transplantation research

Grow your cells in cultureware with Nunc UpCell Surface, reduce the temperature, and harvest your cells.

The Nunc UpCell Surface enables harvesting of cells with high viability and intact cell surface receptors and antigens. You can even harvest cell types that are difficult to detach by other methods, and keep unwanted cell activation to a minimum.

Traditional cell harvesting by enzymatic and mechanical methods often compromises the integrity of surface proteins and the viability of harvested cells. By contrast, Nunc UpCell Surface allows cell harvesting by simply reducing the temperature of the cell culture, resulting in cell populations with preserved cell surface proteins and high cell viability—and there is no need for enzyme removal or inhibition.



Examples of applications using single-cell suspensions harvested by temperature reduction

Cell type	Application	Reference
Macrophages (mouse)	Detachment of cells that are otherwise difficult to detach	Application Note 2
Bone marrow cells and preadipocytes (human)	Cell surface protein preservation (flow cytometry)	Application Note 3
Microglia (rat)	Analysis (detachment and function)	Nakajima et al., 2001
Monocytes and macrophages (human)	Re-seeding/passaging	Collier et al., 2002
Monocytes and macrophages (human)	Activation and analysis (structural)	Gordon and Freedman, 2006
Basophilic cell line RBL-2H3 (rat)	Antigen-mediated degranulation measured by surface plasmon resonance	Yanase et al., 2007

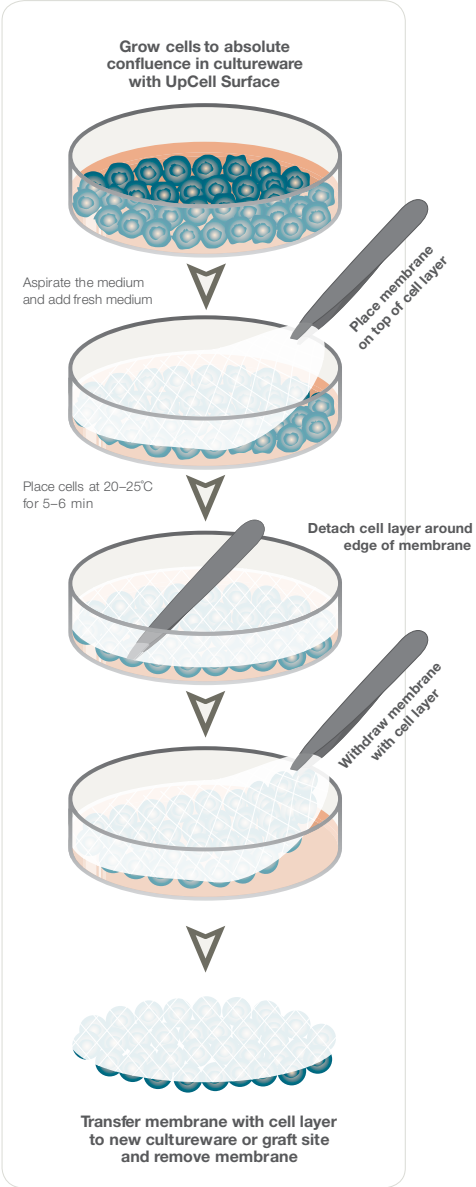
Working with cell sheets

Cell sheets harvested from cultureware with Nunc UpCell Surface can be utilized in the following ways:

- Analyzed *in vitro*, for example, by electron microscopy
- Re-plated to cultureware with Nunc UpCell Surface or traditional cell cultureware
- Used in different transplantation models
- Stacked on top of another cell sheet ("Working with tissue models—cell sheet engineering", see below)

The Nunc UpCell Surface enables harvesting of contiguous cell sheets with preserved cell polarization and held together by normal cell junctions and extracellular matrix. Grow your cells to confluence in cultureware with Nunc UpCell Surface, apply the supplied membrane (only Cat. No. 174901 for 6-well multidish and Cat. No. 174904 for 3.5 cm dish), reduce the temperature, and harvest the cell sheet.

Anchorage-dependent cells in culture initially attach to proteins adsorbed to the cultureware from the medium, and produce and deposit a subcellular matrix during the course of the cultivation (Brevig et al., 2006; Kushida et al., 1999; Pompe et al., 2003). Cells in culture are held together by cell junctions and the matrix deposited by the cells. Traditional enzymatic or mechanical harvesting disrupts these cell-to-cell and cell-to-matrix contacts, as well as the subcellular matrix, and destroys the integrity and polarization of the culture. With the Nunc UpCell Surface, cells can be detached as contiguous sheets from the cultureware. The subcellular matrix and the supplied membrane provides the mechanical strength necessary for the handling of the detached cell sheet.



Examples of applications using cell sheets harvested by temperature reduction

Cell type	Application	Reference
Aortic endothelial cells (cow)	Analysis (structural and matrix deposition)	Kushida et al., 1999
Keratinocytes (human)	Analysis (electron microscopy)	Yamato et al., 2001
Urothelial cells (human)	Analysis (electron microscopy)	Shiroyanagi et al., 2003
Retinal pigment epithelial cell line ARPE-19 (human)	Analysis (light microscopy)	Kubota et al., 2006
Kidney epithelial cells (human and dog)	Re-plating to traditional cultureware and analysis (electron and fluorescence microscopy)	Application Note 4 Kushida et al., 2005
Lung cells (rat)	Re-plating to traditional cultureware and analysis (fluorescence microscopy)	Nandkumar et al., 2002
Smooth muscle cells and fibroblasts (human)	Re-plating to PIPAAm surface and analysis (functional) and transplantation	Hobo et al., 2008
Mesenchymal stem cells and skin fibroblasts (rat)	Analysis (structural and functional) and transplantation	Miyahara et al., 2006
Corneal stem cells (human and rabbit)	Analysis (structural) and transplantation	Nishida et al., 2004
Corneal endothelial cells (human)	Analysis (structural and functional) and transplantation	Sumide et al., 2006
Oral mucosal epithelial cells (dog)	Analysis (structural) and transplantation	Ohki et al., 2006
Tracheal epithelial cells (rabbit)	Transplantation	Kanzaki et al., 2006
Periodontal ligament cells (human)	Transplantation	Hasegawa et al., 2005

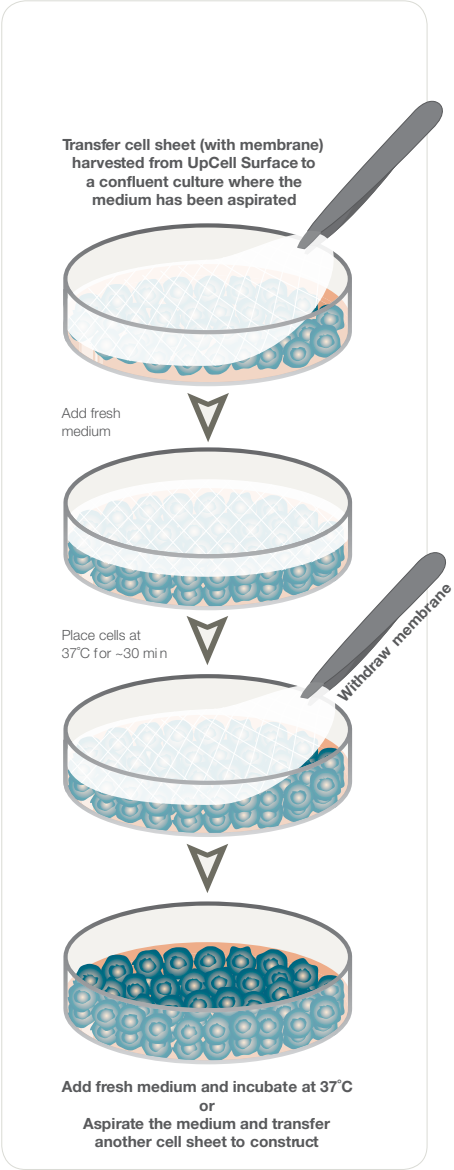
Working with tissue models—cell sheet engineering

Cell sheet constructs prepared in cultureware with Nunc UpCell Surface can be utilized in the following ways:

- Analyzed *in vitro*, for example, by functional tissue-specific tests
- Cultivated *in vitro*, for example, as 3D co-cultures
- Used in different transplantation models, where cell sheets are stacked before or during the transplantation procedure

The Nunc UpCell Surface enables harvested cell sheets to be stacked in order to form 3D tissue models. Grow your cells to confluence in cultureware with Nunc UpCell Surface, harvest the cell sheet, and transfer the cell sheet to another cell sheet. No scaffold is needed.

Stacking of cell sheets, also known as cell sheet engineering, was pioneered by Okano and colleagues (Yamada et al., 1990; Yang et al., 2005 and 2007). The preserved subcellular matrix of a harvested cell sheet provides the adhesive necessary for stacking. It functions as a natural glue to bond the cell sheet to an underlying recipient cell sheet or to a recipient site in a transplantation model, without the use of fibrin glue or sutures.



Examples of cell sheet engineering using cell sheets harvested by temperature reduction

Cell type	Application	Reference
Aortic endothelial cells (human) and hepatocytes (rat)	Cultivation (3D co-culture) and analysis (structural)	Harimoto et al., 2002
Hepatocytes (mouse and human)	Analysis (structural and functional) and stacking during transplantation	Ohashi et al., 2007
Skeletal myoblast (dog)	Analysis (structural) and stacking during transplantation	Hata et al., 2006
Lung and skin fibroblasts (rat)	Analysis (structural) and stacking during transplantation	Kanzaki et al., 2007
Cardiomyocytes (rat)	Cultivation and analysis (structural and functional) and stacking before transplantation	Sekine et al., 2006; Sekiya et al., 2006; Shimizu et al., 2002 and 2006

Quality assurance

Quality is inherent in our culture. From product development and sourcing raw materials to manufacturing and customer service, quality is reflected in every Thermo Scientific™ Nunc™ product.

A certificate of quality is packed in each box of cultureware with Nunc UpCell Surface. This certificate is your confirmation that the product has been validated according to the following tests:

Cell growth

Each manufacturing lot is sampled and subjected to performance testing for growth with the 3T3-Swiss Albino cell line (derived from a mouse embryo fibroblast) in accordance with standard operating procedures. Acceptance level: minimum of 80% confluence.

Cell detachment

The manufacturing lot is sampled and subjected to performance testing for cell detachment by temperature reduction with the 3T3-Swiss Albino cell line in accordance with standard operating procedures. Adherent cells are detached by temperature decreasing treatment (below 32°C) and the degree of detachment is measured. Acceptance level: detachment of 50% or more of the cells.

Sterility

Sterility is obtained by using ethylene oxide gas according to ISO 11135-1 guidelines (Sterilization of health care products, Ethylene oxide, Part 1: Requirements for development, validation, and routine control of a sterilization process for medical devices).

Application Note 1

Advantages of Nunc 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 (Huang et al., 2010). 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 Nunc UpCell Surface, which transitions from hydrophobic to hydrophilic as the 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 Nunc 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 cell 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 the 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 the 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 Nunc 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 the dark. Invitrogen™ eBioscience™ Fixable Viability Dye eFluor™ 450 (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 Nunc UpCell Surfaces

MSCs grown on the Nunclon Delta and Nunc 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 Nunc UpCell Surface (Figure 2A). There was no significant difference in cell viability between the two dissociation methods (Figure 2B).

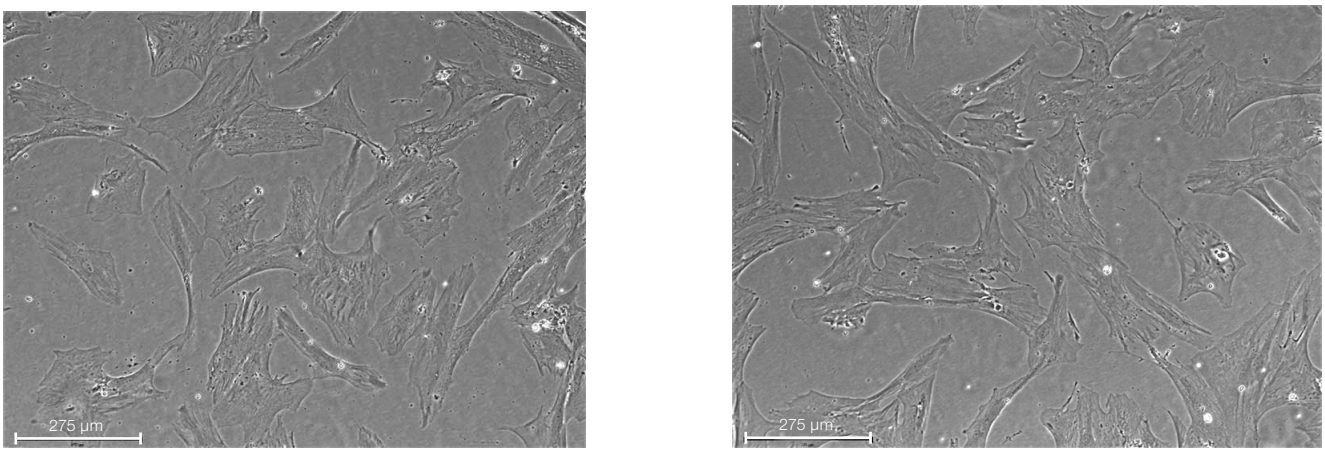


Figure 1. Phase-contrast images of MSCs grown on Nunclon Delta and Nunc UpCell Surfaces. Images were captured on the Invitrogen™ EVOS™ M7000 Imaging System at 10x magnification (scale bar: 275 µm).

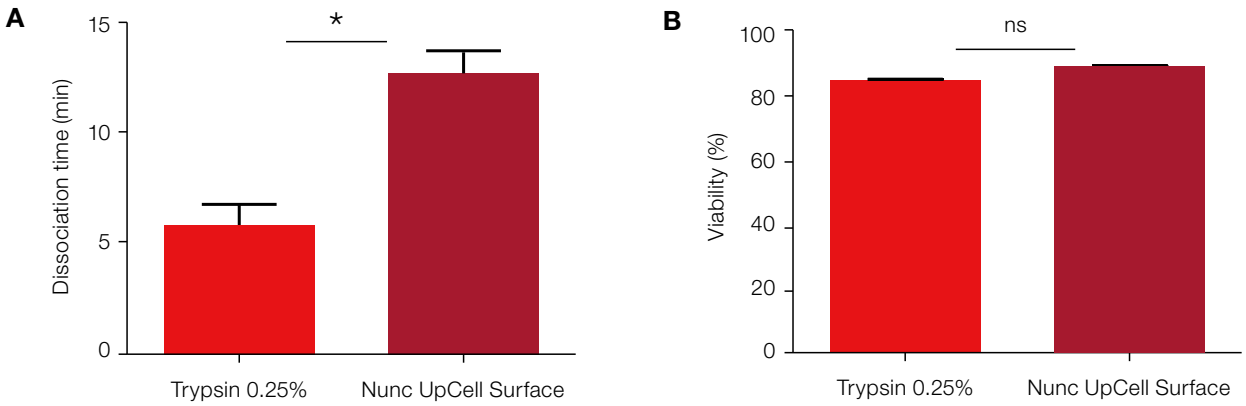


Figure 2. Despite longer dissociation time, the Nunc UpCell Surface does not impact cell viability. MSCs were treated with trypsin on the Nunclon Delta Surface or dissociated by temperature shift on the Nunc UpCell Surface until cells were completely detached. **(A)** Dissociation time was noted, and **(B)** cell viability was measured using the Countess II Automated Cell Counter. Individual experiments were done in duplicate, and data are represented as the 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 (Tsuji et al., 2017 and Chaudhry, 2008). We examined three cell surface antigens expressed on MSCs, i.e., 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 (Wilkins et al., 1999). Thus, we wanted to determine if the use of the Nunc 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 Invitrogen™ Attune™ NxT Flow Cytometer.

Post-acquisition analysis indicated that the expression of CD44 was reduced by cell dissociation using trypsin treatment, compared to the Nunc UpCell Surface (Figure 3, left panels). The Nunc 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 Nunc 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 Nunc UpCell Surface has milder effects on cultured MSCs, preserving cell surface antigens and maintaining high viability compared to trypsin-mediated dissociation.

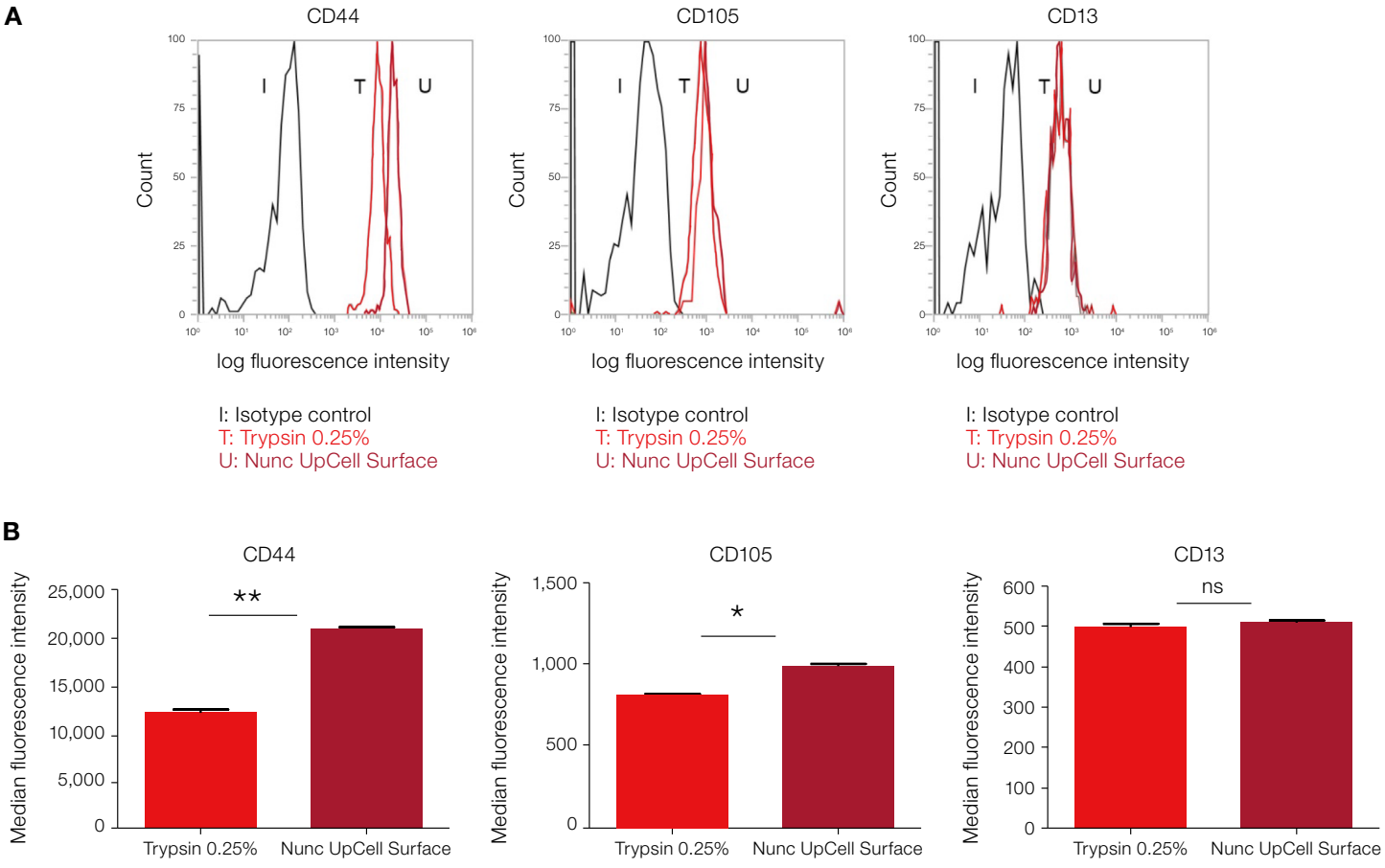


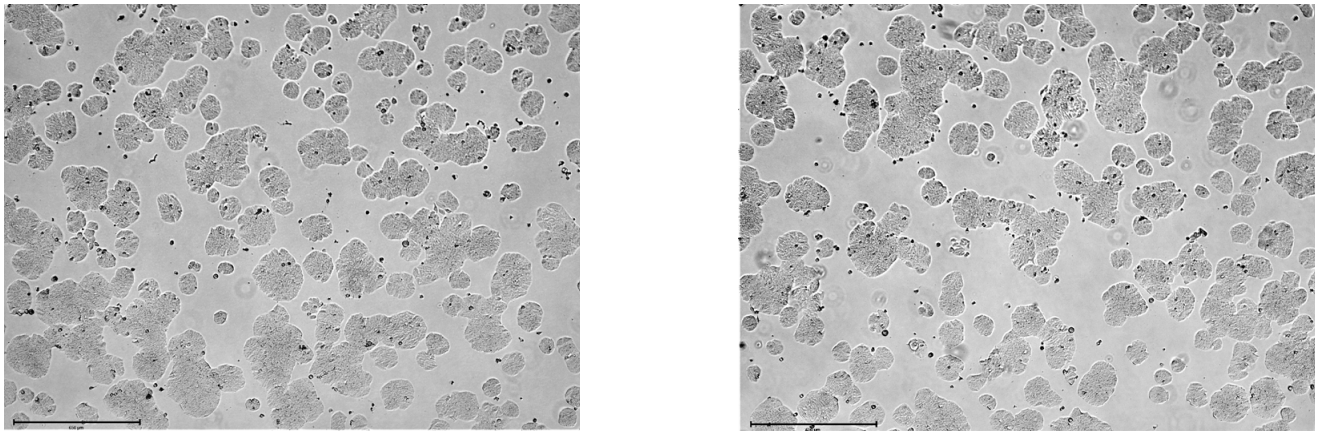
Figure 3. Measurement of CD44, CD105, and CD13 fluorescence intensity in MSCs after antibody staining. (A) Representative flow cytometry histograms showing expression of CD44, CD105, and CD13 following dissociation using 0.25% trypsin or the Nunc UpCell Surface. **(B)** Median fluorescence intensities of surface antigens are demonstrated as bar graphs. Individual experiments were done in duplicate, and data are represented as the 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 Nunc UpCell Surfaces

HT-29 cells were grown on the Nunclon Delta and Nunc UpCell Surfaces. As was the case with MSCs, the Nunc 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 **Nunc UpCell Surface**

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

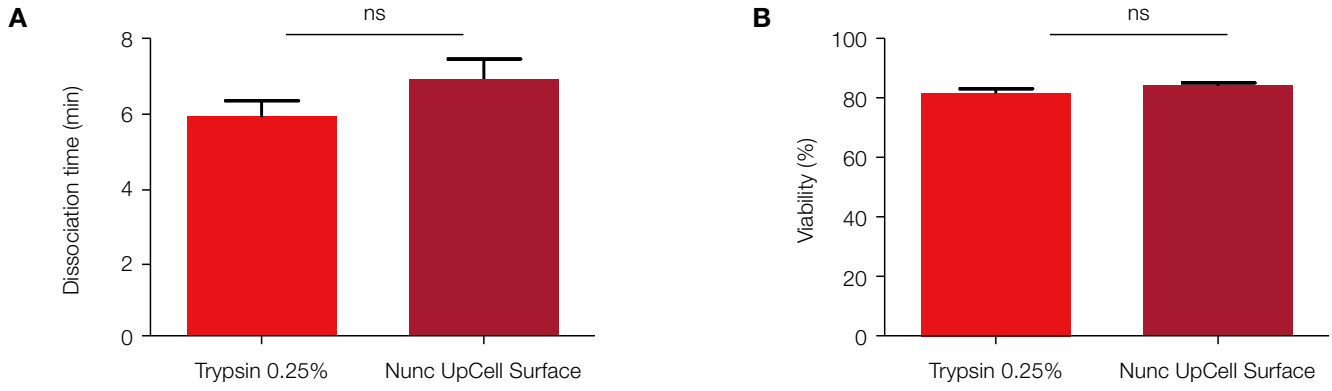


Figure 5. Despite a slight difference in dissociation time, the Nunc UpCell Surface does not impact cell viability. HT-29 cells were treated with trypsin on the Nunclon Delta Surface or dissociated by temperature shift on the Nunc UpCell Surface until cells were completely detached. **(A)** Dissociation time was noted, and **(B)** cell viability was measured using the Countess II Automated Cell Counter. Each 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 Nunc UpCell Surface. Following dissociation, HT-29 cells were stained with eBioscience CD44 Monoclonal Antibody, 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 Nunc UpCell Surfaces

RAW 264.7 cells were plated on Nunclon Delta and Nunc 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).

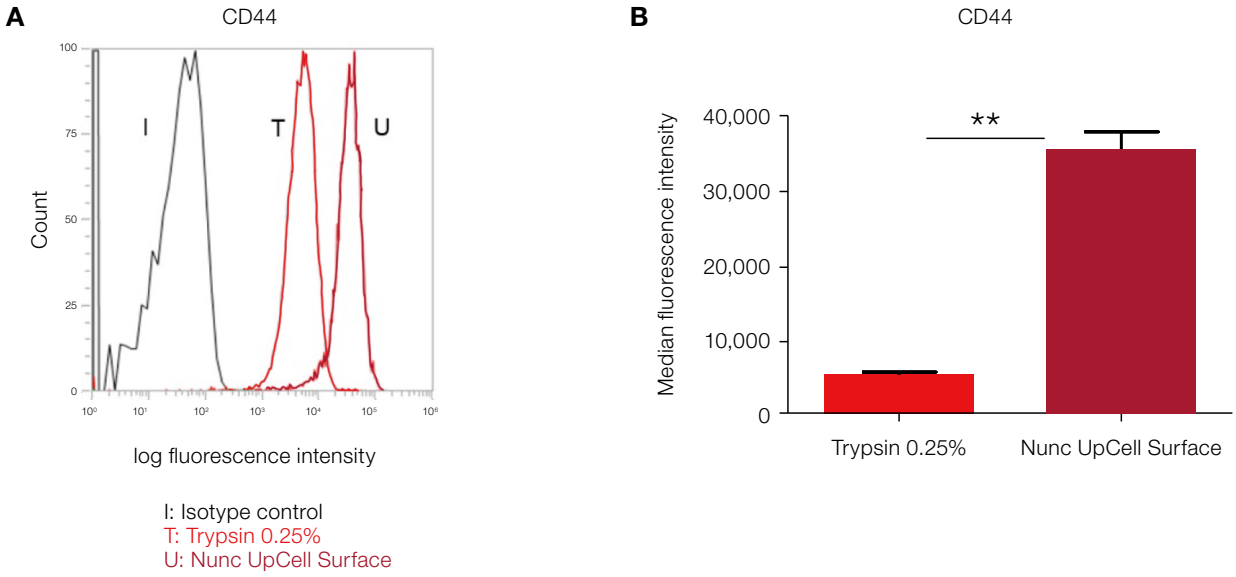
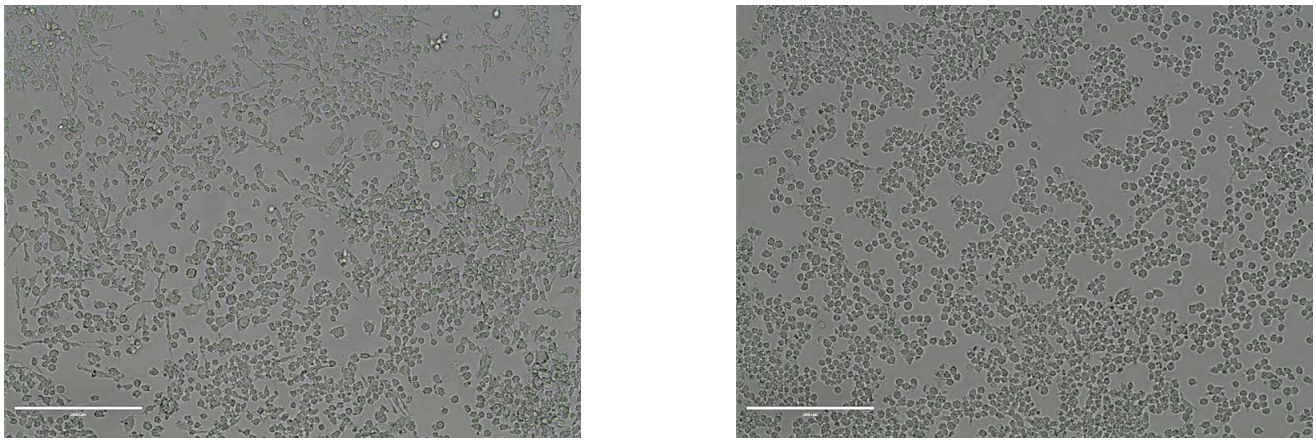


Figure 6. Measurement of CD44 fluorescence intensity in HT-29 cells after antibody staining. (A) Representative flow cytometry histograms showing expression of CD44 following dissociation using 0.25% trypsin or the Nunc UpCell Surface. **(B)** Median fluorescence intensity of CD44 surface antigen is 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 Nunc UpCell Surface

Figure 7. Brightfield images of RAW 264.7 cells grown on Nunclon Delta and Nunc 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 Nunc UpCell Surface required only 15 min at 4°C (Figure 8A). Viability of cells harvested from the Nunc 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 Nunc UpCell Surface. Following dissociation, cells were stained with Invitrogen™ eBioscience™ antibodies against CD11b (Cat. No. 11-0112-82) and CD44, 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 Nunc UpCell Surface (Figure 9). Taken together, our results indicate that dissociation via the Nunc UpCell Surface offers significant improvements for firmly adherent cell types like RAW 264.7. Compared to trypsin, the Nunc UpCell Surface drastically reduced dissociation time, maintained higher viability, and preserved critical cell surface antigens.

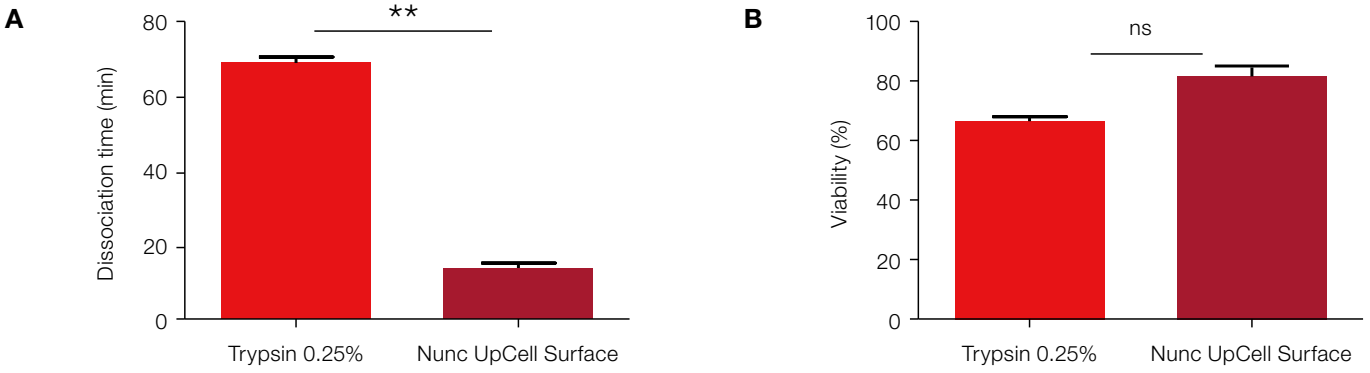


Figure 8. Faster dissociation of RAW 264.7 cells from the Nunc UpCell Surface. RAW 264.7 cells were treated with trypsin on the Nunclon Delta Surface or dissociated at 4°C on the Nunc UpCell Surface until cells were completely detached. **(A)** Dissociation time was noted, and **(B)** cell viability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

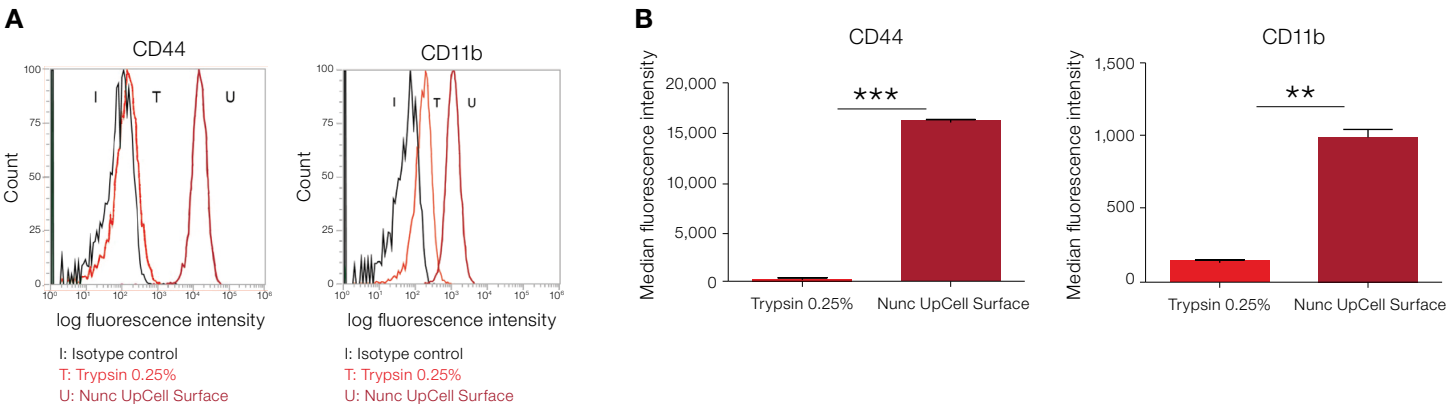


Figure 9. Measurement of CD44 and CD11b fluorescence intensity in RAW 264.7 cells after antibody staining. (A) Representative flow cytometry histograms showing expression of CD44 and CD11b following dissociation using 0.25% trypsin or the Nunc UpCell Surface. **(B)** Median fluorescence intensities of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

Conclusion

These results indicate that the Nunc 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 Nunc UpCell Surface, suggesting this surface is an especially effective option for sticky cell lines.

Use of the Nunc 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.

Application Note 2

Nunc UpCell Surface versus trypsinization and scraping in cell detachment

Cells that are difficult to detach from traditional cultureware by enzymatic or mechanical methods may be harvested from cultureware with the Nunc UpCell Surface simply by reducing the temperature of the cell culture. This application note compares the recovery of mouse peritoneal macrophages harvested from the Nunc UpCell Surface using temperature reduction with those harvested from traditional cultureware (tissue culture-treated polystyrene) using trypsinization or scraping.

Methods

Mouse peritoneal macrophages in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) were seeded in one dish with the Nunc UpCell Surface and two traditional cultureware dishes at 2.4 x 10^5 cells/cm^2. The cells were incubated at 37°C in a humidified atmosphere of 5% CO_2 in air. After 2 hours of incubation, nonadherent cells were removed by washing with phosphate-buffered saline (PBS). Cells were then cultured for 2 days in RPMI 1640 medium supplemented with 10% FBS and harvested using one of the following procedures:

Harvest of cells from the Nunc UpCell Surface using temperature reduction

- Nonadherent cells were removed by washing with Ca^{2+}- and Mg^{2+}-free PBS
- 4.0 mL RPMI 1640 medium supplemented with 10% FBS was added, and the dish was incubated at 20°C for 30 min
- Detached cells were harvested

Harvest of cells from traditional cultureware using trypsinization

- Nonadherent cells were removed by washing with Ca^{2+}- and Mg^{2+}-free PBS
- 1.0 mL of 0.25% trypsin/EDTA was added, and the dish was incubated at 37°C for 5 min
- 3.0 mL RPMI 1640 medium supplemented with 10% FBS was added
- Detached cells were harvested

Harvest of cells from traditional cultureware using EDTA and scraping

- Nonadherent cells were removed by washing with Ca^{2+}- and Mg^{2+}-free PBS
- 4.0 mL of 2.5 mM EDTA/PBS was added, and the dish was incubated on ice for 20 min
- The cells were detached by scraping and harvested

The harvested cells were counted and the recovery ratio was calculated.

Results

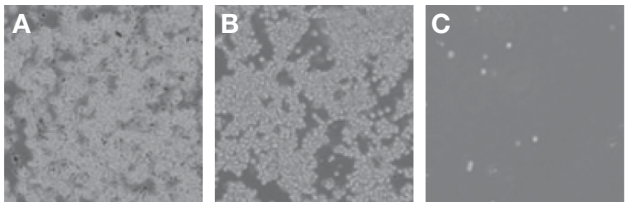


Figure 1. Photomicrographs of mouse peritoneal macrophages on the Nunc UpCell Surface (A) before and (B) after temperature reduction. After temperature reduction, the cells detached from the surface and became spherical. **(C)** After harvesting of the cells by pipetting, only a few cells remained on the Nunc UpCell Surface.

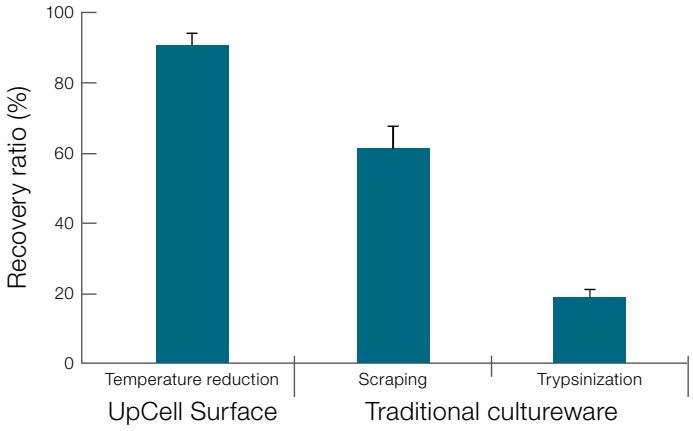


Figure 2. Recovery ratio of mouse peritoneal macrophages harvested from the Nunc UpCell Surface compared with recovery ratios of these cells harvested by either enzymatic (trypsinization) or mechanical (scraping) methods. The recovery of cells from the Nunc UpCell Surface was significantly higher than the recovery of cells harvested from traditional cultureware by trypsinization or scraping. Mean and SD are shown.

Nunc UpCell Surface versus trypsinization in preservation of surface proteins during cell harvesting

Enzymatic cell harvesting often compromises the integrity of cell surface proteins. By contrast, the UpCell Surface allows cell harvesting simply by reducing the temperature of the cell culture, resulting in cell populations with preserved cell surface proteins. This application note compares the integrity of CD140a (a cell surface tyrosine kinase receptor for members of the platelet-derived growth factor family) on human bone marrow cells and preadipocytes harvested from the Nunc UpCell Surface by temperature reduction and from traditional cultureware (tissue culture-treated polystyrene) by trypsinization. The cells were stained using a phycoerythrin (PE)-conjugated antibody against human CD140a and subsequently analyzed by flow cytometry.

Methods

Human bone marrow cells or human preadipocytes in DMEM supplemented with 10% FBS were seeded at 6.8 x 10^3 cells/cm^2 in a 6 cm dish with the Nunc UpCell Surface and also in a traditional 6 cm dish. Cells were incubated for 24 hours at 37°C in a humidified atmosphere of 5% CO_2 in air, then harvested using one of the following procedures:

Harvest of cells from the Nunc UpCell Surface using temperature reduction

- The dish was incubated at 20°C for 30 min (no change of culture medium)
- Detached cells were transferred to a 15 mL conical-bottom tube

Harvest of cells from traditional cultureware using trypsinization

- Cells were gently washed once with Ca^{2+}- and Mg^{2+}-free PBS
- 2.0 mL of 0.25% trypsin/EDTA was added, and the dish was incubated at 37°C for 3 min
- 10 mL of culture medium was then added to the dish, and the cells were transferred to a 15 mL conical-bottom tube

Cells harvested by temperature reduction or trypsinization were washed twice by centrifugation (300 x *g*, 5 min). Supernatants were discarded, and aliquots of 5.0 x 10^5 cells were incubated with 200 µL of PE-conjugated mouse monoclonal antibody against human CD140a (5 µg/mL; BD Pharmingen, NJ, USA) or PE-conjugated mouse IgG2a isotype-control antibody (5 µg/mL; BD Pharmingen). After incubation at 4°C for 60 min, cells were washed with PBS and analyzed using a FC500 Flow Cytometer (Beckman Coulter, CA, USA).

Results

Human bone marrow cells and preadipocytes harvested from the Nunc UpCell Surface by temperature reduction had preserved cell surface CD140a, whereas CD140a on cells harvested from traditional cultureware by a short (3 min) trypsinization could barely be detected (Figure 1). This demonstrates that using the Nunc UpCell Surface and temperature reduction preserves the integrity of cell surface proteins to a higher degree than using traditional cultureware and enzymatic cell harvesting.

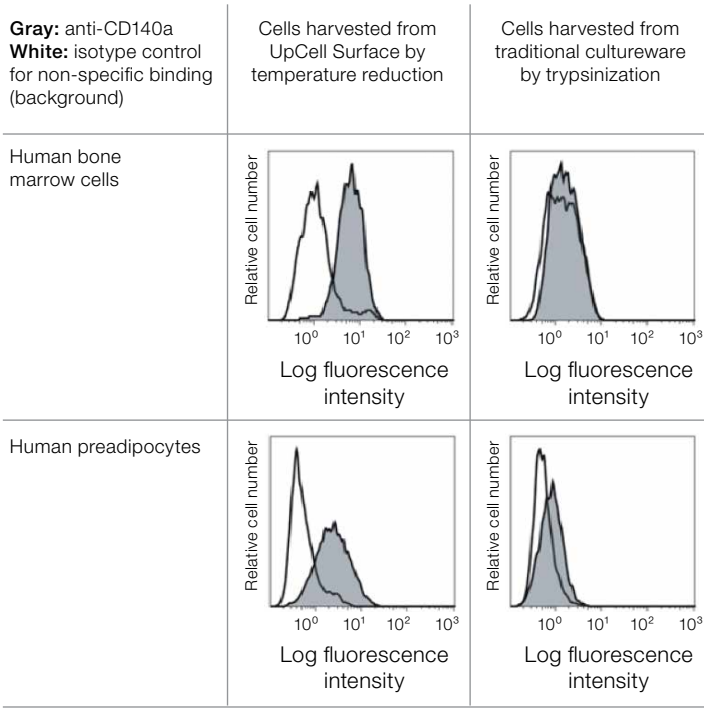


Figure 1. The Nunc UpCell Surface preserves the integrity of CD140a.

Transfer of a cell sheet with preserved polarization and cell-cell junctions

A contiguous cell sheet can be harvested from the Nunc UpCell Surface without compromising the subcellular protein matrix and cell-cell junctions. In this application note, the transfer of a cell sheet from the Nunc UpCell Surface to a cell culture insert is described, and the integrity of cell-cell junctions of the transferred cell sheet is demonstrated. This application note is based on Kushida et al. (2005) "A noninvasive transfer system for polarized renal tubule epithelial cell sheets using temperature-responsive culture dishes". *European Cells and Materials* 10:23–30.

Methods

Madin-Darby Canine Kidney (MDCK) cells in DMEM supplemented with 10% FBS, penicillin (100 units/mL), and streptomycin (100 µg/mL) were seeded at 5.7 x 10^4 cell/cm^2 in a 3.5 cm dish with the Nunc UpCell Surface. The cells were incubated for 21 days at 37°C in a humidified atmosphere of 5% CO_2 in air. The cells were then harvested as an intact sheet:

- The dish was placed at 20°C for 60 min
- A supporting membrane was placed on top of the cell layer, and the culture medium was aspirated
- The edge of the membrane was gently released from the dish using forceps, and the membrane with the attached cell layer was then transferred to a cell culture insert and incubated at 20°C for 30 min
- Sufficient culture medium was added to cover the cell sheet and membrane, and the membrane was removed from the cell layer using forceps

Results

A layer of MDCK cells (Figure 1A) was detached from the Nunc UpCell Surface by temperature reduction and harvested as a single contiguous cell sheet using a membrane as a carrier. After harvesting, no cells were observed on the Nunc UpCell Surface (Figure 1B). The harvested cell sheet was transferred to a cell culture insert, and adhered readily (Figure 1C).

The cell sheet was examined by transmission electron microscopy after transfer. Tight junctions (Figure 2, arrowheads) were intact and the cell layer had maintained its apical-basal polarity.

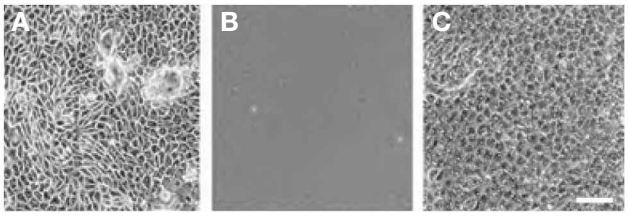


Figure 1. Phase-contrast microscopy of MDCK cells before and after cell sheet transfer. Scale bar, 100 µm. Printed with permission from *European Cells and Materials* (ecmjournal.org).

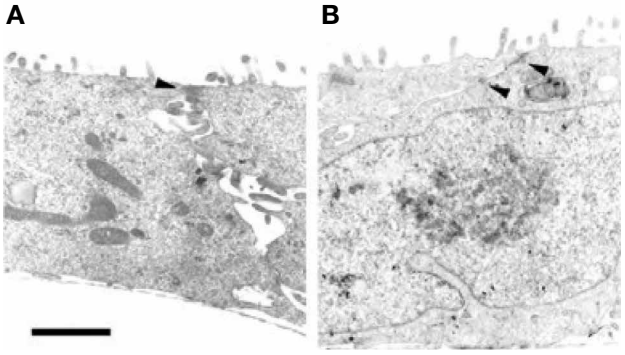


Figure 2. Transmission electron microscopy of a MDCK cell sheet transferred to a cell culture insert. (A) Cell layer immediately after transfer. **(B)** Cell layer 5 hours after transfer. Scale bar, 1 µm. Printed with permission from *European Cells and Materials* (ecmjournal.org).

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Ordering information

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

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