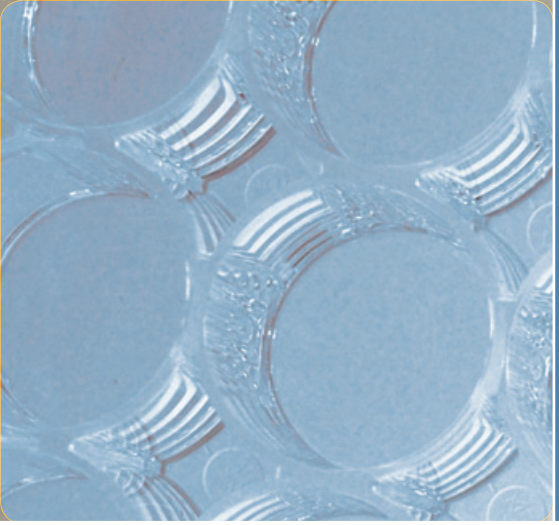
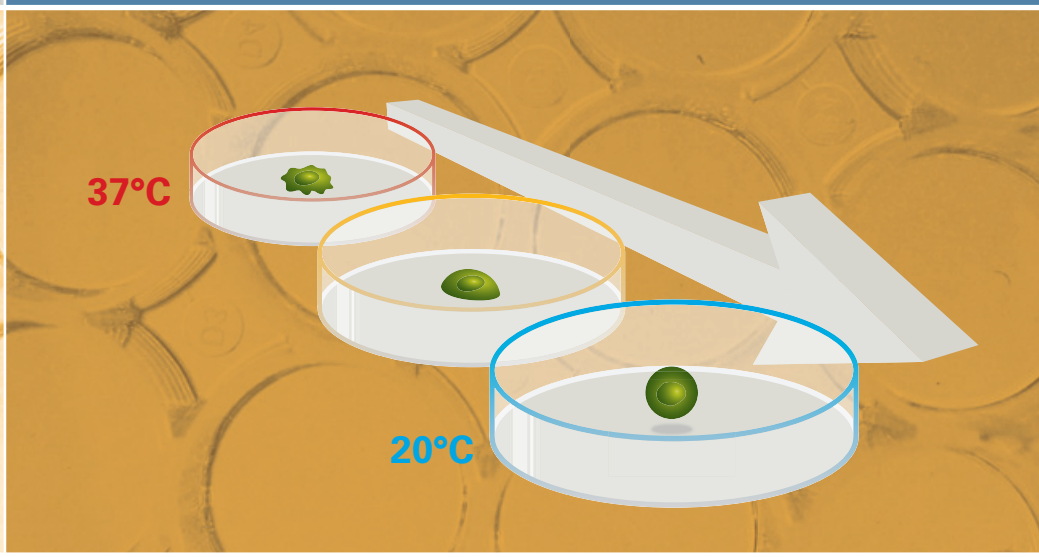


Cell Harvesting by Temperature Reduction



Thermo Scientific Nunc UpCell Surface

nunc™

Thermo SCIENTIFIC

Features and Benefits

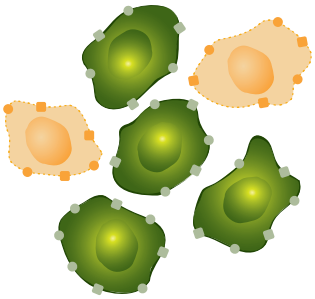
Preserving Cell Surface Proteins

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.

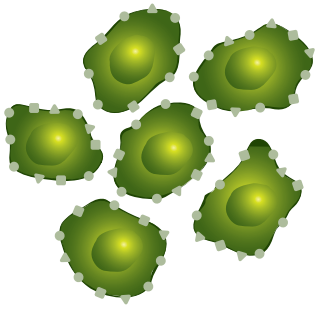
- No trypsin – preserves cell surface proteins
- No scraping – get high cell viability
- Minimal hands-on time
- Quick, clean and simple - just reduce the temperature

Cell harvesting using enzymatic digestion, such as trypsinization, results in degradation of cell surface proteins. 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 harvested from UpCell Surface by temperature reduction



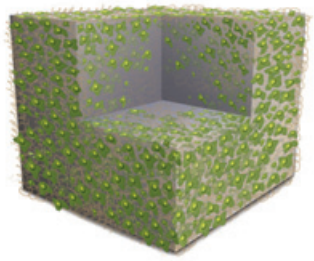
Creating 3D Tissue Models

The 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.

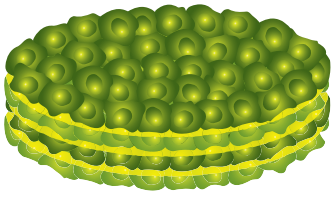
- No scaffold – make 3D tissue models without exogenous material
- No uneven cell distribution – control the spatial distribution of cells in 3D
- Endless possibilities for mixing cell types and creating 3D co-cultures
- Just harvest and stack cell sheets

In tissue engineering, three-dimensional (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, poly lactic acid (PLA), poly glycolic 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



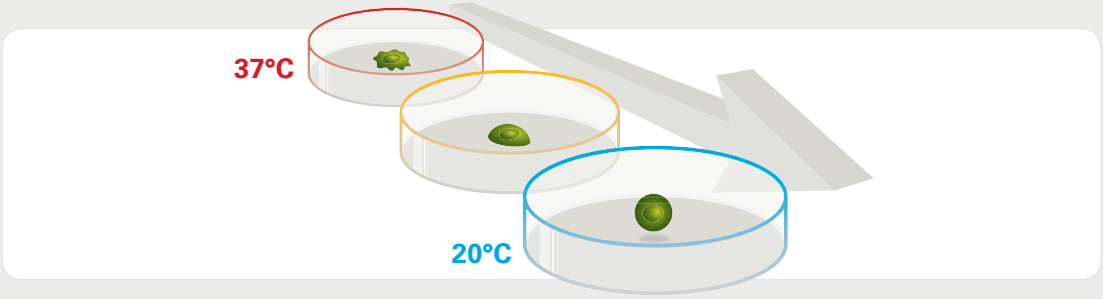
Tissue engineered using UpCell Surface



Thermo Scientific Nunc UpCell Surface

Temperature-Responsive Cell Culture Surface

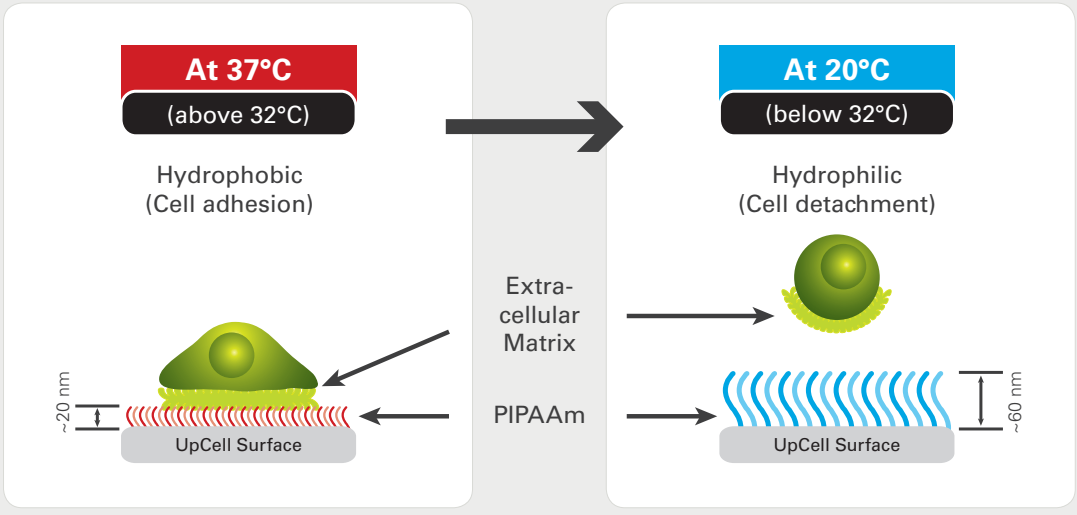
UpCell Surface is designed to respond to changes in temperature. It releases adherent cells by a simple reduction of the temperature of the cell culture. Products with UpCell Surface include Thermo Scientific Nunc MicroWell plates, multidishes and dishes.



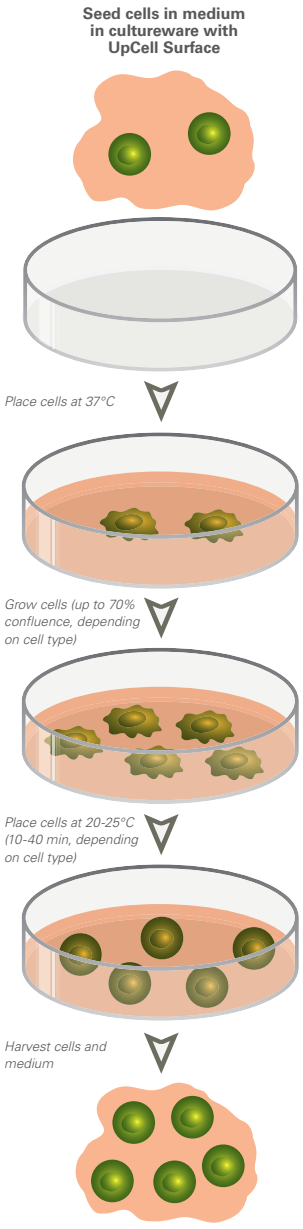
The covalently immobilized polymer poly(N-isopropylacrylamide), or PIPAAm, forms an even and thin layer on the cultureware. The PIPAAm layer is slightly hydrophobic at 37°C, allowing cells to attach and grow. When the temperature of the culture is reduced to below 32°C, the PIPAAm layer becomes very 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 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 Thermo Scientific Nunc UpCell Surface can be

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

Grow your cells in cultureware with UpCell Surface, reduce the temperature, and harvest your cells. It is that simple!

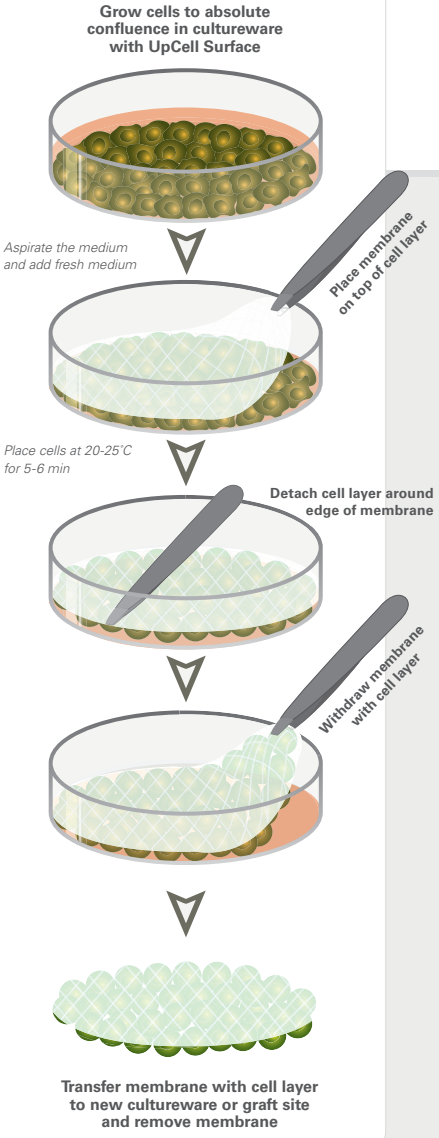
The Nunc UpCell Surface enables harvesting of cells with high viability and intact cell surface receptors and antigens. Even harvest cell types that are difficult to detach by other methods, and keep unwanted cell activation to a minimum.

Traditional cell harvesting by enzymatic and mechanical methods often compromises the integrity of surface proteins and the viability of harvested cells. By contrast, 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 1
Bone marrow cells & preadipocytes (human)	Cell surface protein preservation (flow cytometry)	Application Note 2
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 & 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 Thermo Scientific Nunc UpCell Surface can be

- Analyzed *in vitro*, for example, by electron microscopy
- Re-plated to cultureware with UpCell Surface or traditional cell cultureware
- Used in different transplantation models
- Stacked on top of another cell sheet (see next section, Tissue Models - Cell Sheet Engineering)

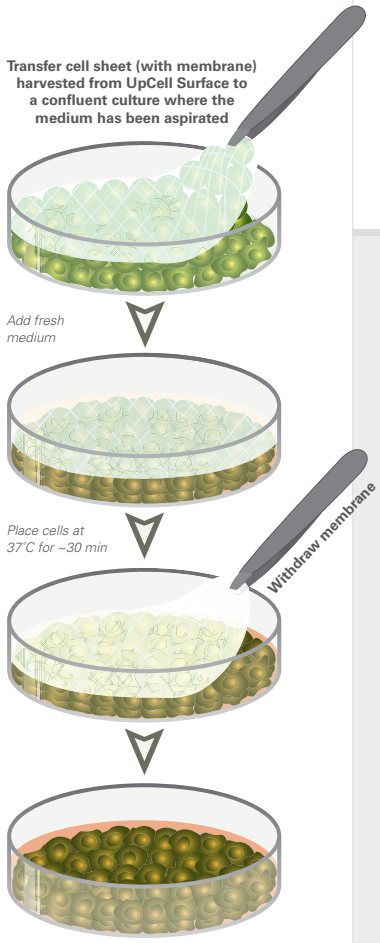
The UpCell Surface enables harvesting of contiguous cell sheets with preserved cell polarization and held together by normal cell junctions and extracellular matrix. Grow your cells to confluence in cultureware with UpCell Surface, apply the supplied membrane (only Cat. Nos. 174901 6 well multidish and 174904 3.5 cm dish), reduce the temperature, and harvest the cell sheet. It is that easy!

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 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 & analysis (electron and fluorescence microscopy)	Application Note 3 Kushida et al., 2005
Lung cells (rat)	Re-plating to traditional cultureware & analysis (fluorescence microscopy)	Nandkumar et al., 2002
Smooth muscle cells & fibroblasts (human)	Re-plating to PIPAAm surface & analysis (functional) & transplantation	Hobo et al., 2008
Mesenchymal stem cells & skin fibroblasts (rat)	Analysis (structural and functional) & transplantation	Miyahara et al., 2006
Corneal stem cells (human and rabbit)	Analysis (structural) & transplantation	Nishida et al., 2004
Corneal endothelial cells (human)	Analysis (structural and functional) & transplantation	Sumide et al., 2006
Oral mucosal epithelial cells (dog)	Analysis (structural) & 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 Thermo Scientific Nunc UpCell Surface can be

- 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 UpCell Surface enables harvested cell sheets to be stacked in order to form 3D tissue models. Grow your cells to confluence in cultureware with 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) & hepatocytes (rat)	Cultivation (3D co-culture) & analysis (structural)	Harimoto et al., 2002
Hepatocytes (mouse and human)	Analysis (structural and functional) & stacking during transplantation	Ohashi et al., 2007
Skeletal myoblast (dog)	Analysis (structural) & stacking during transplantation	Hata et al., 2006
Lung and skin fibroblasts (rat)	Analysis (structural) & stacking during transplantation	Kanzaki et al., 2007
Cardiomyocytes (rat)	Cultivation & analysis (structural and functional) & 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 Thermo Scientific Nunc UpCell Surface.
This certificate is your guarantee 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 (under 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 (Sterilization of health care products, Ethylene oxide, Part 1: Requirements for development, validation and routine control of a sterilization process for medical devices).

Non-pyrogenic
Representative samples of products with UpCell Surface are tested according to the principles of the LAL-test described in the FDA guidelines and certified nonpyrogenic with a documented endotoxin level of less than 20 endotoxin units/device (0.5 Endotoxin units/mL) as stated in the USP.

Toxicity
The material has successfully passed the USP biological reactivity Class VI test - 50°C (7 days implant). Cytotoxicity test according to ISO 10993-5: Biological evaluation of medical devices - Part 5: Tests for *in vitro* cytotoxicity.

Products

Cultureware with UpCell Surface
With lid. Sterile

Cat. No.	174897	174898	174899	174900	174901	174902	174905	174903	174906	174904
Format	96 MicroWell Plate w/ flat bottom	48 well Multidish	24 well Multidish	12 well Multidish	6 well Multidish	10 cm Dish	10 cm Dish with grid	6 cm Dish	6 cm Dish with grid	3.5 cm Dish
Number of wells	96	48	24	12	6	1	1	1	1	1
Culture area, cm^2/well	0.33	1.1	1.9	3.5	9.6	56.7	56.7	21.5	21.5	8.8
Max. external dimensions, mm	128x86	128x86	128x86	128x86	128x86	92x17	92x17	60x15	60x15	40x12
Suggested working volume, mL/well	0.2	0.5	1	2	3	12.5	12.5	5	5	3
Airvent	+	+	+	+	+	+	+	+	+	+
Units per pack/case	1/8	1/6	1/6	1/6	1/6	1/6	1/6	5/30	5/30	5/30

Cultureware with UpCell Surface is intended for research purposes only and single-use only. Any other use is not warranted by Thermo Fisher Scientific. Do not use the product for clinical or diagnostic purposes.
UpCell Surface is licensed from CellSeed Inc. Made in Japan.

Application Note 1

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 UpCell Surface simply by reducing the temperature of the cell culture.
This application note compares the recovery of mouse peritoneal macrophages harvested from the 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 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, non-adherent 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 UpCell Surface using temperature reduction

- Non-adherent 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

- Non-adherent 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

- Non-adherent 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

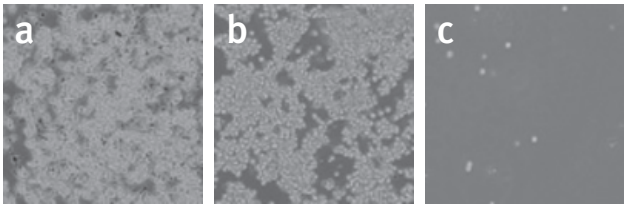


Figure 1. Photomicrographs of mouse peritoneal macrophages on the UpCell Surface before (a) and after (b) temperature reduction. After temperature reduction, the cells detached from the surface and became spherical. After harvesting of the cells by pipetting, only a few cells remained on the UpCell Surface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

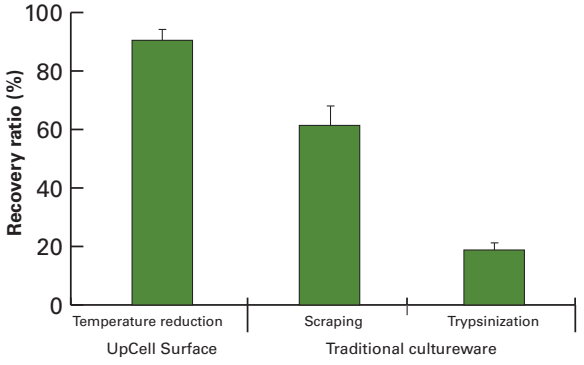


Figure 2. Recovery ratio of mouse peritoneal macrophages harvested from the UpCell Surface was compared with recovery ratios of these cells harvested by either enzymatic (trypsinization) or mechanical (scraping) methods. The recovery of cells from the UpCell Surface was significantly higher than the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

Application Note 2

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 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 Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) were seeded at 6.8 x 10^3 cells/cm^2 in a 6 cm dish with 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 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

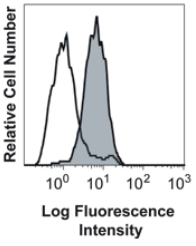
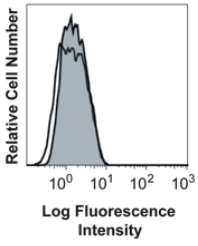
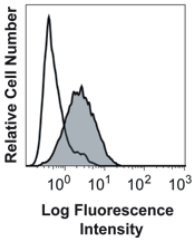
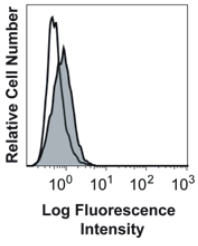
- Cells were gently washed once with Ca^{2+}– and Mg^{2+}–free phosphate-buffered saline (PBS)
- 2.0 mL of 0.25% trypsin/EDTA was added, and the dish was incubated at 37°C for 3 min.
- 10 mL culture medium was then added to the dish, and the cells were transferred to a 15 mL conical-bottom tube

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 IgG_{2a} isotype-control antibody (5 µg/mL; BD Pharmingen). After incubation at 4°C for 60 min. cells were washed with PBS and analyzed by flow cytometry (FC500; Beckman Coulter, CA, USA).

Results

Human bone marrow cells and preadipocytes harvested from the 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. This demonstrates that using UpCell Surface and temperature reduction preserves the integrity of cell surface proteins to a higher degree than using traditional cultureware and enzymatic cell harvesting.

UpCell Surface Preserves the Integrity of CD140a

Grey: anti-CD140a **White:** isotype control for non-specific binding (background)	Cells harvested from UpCell Surface by temperature reduction	Cells harvested from traditional cultureware by trypsinization
Human bone marrow cells		
Human preadipocytes		

Application Note 3

Transfer of Cell Sheet with Preserved Polarization and Cell-Cell Junctions

A contiguous cell sheet can be harvested from the UpCell Surface without compromising the subcellular protein matrix and cell-cell junctions. In this application note, the transfer of a cell sheet from the 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., *A non-invasive transfer system for polarized renal tubule epithelial cell sheets using temperature-responsive culture dishes.* European Cells and Materials 2005; 10:23-30.

Methods

Madin-Darby Canine Kidney (MDCK) cells in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (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 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 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 UpCell Surface (Figure 1b). The harvested cell sheet was transferred to a cell culture insert, and adhered readily (Figure 1c).

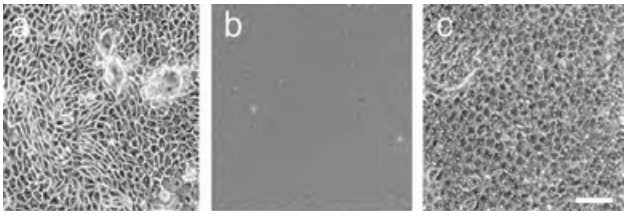


Figure 1. Phase contrast microscopy of MDCK cells before and after cell sheet transfer. Scale bar, 100 µm. Printed with permission from European Cells and Materials (ecmjournal.org).

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

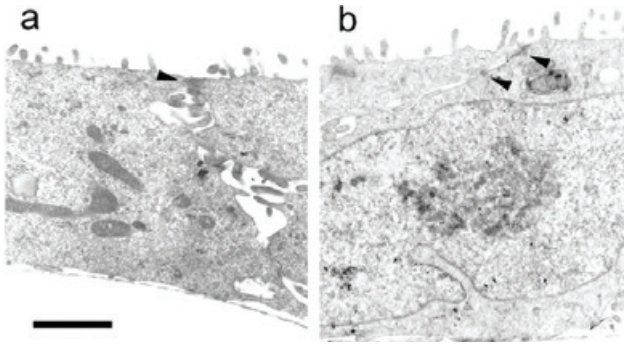


Figure 2. Transmission electron microscopy of MDCK cell sheet transferred to a cell culture insert. a: Cell layer immediately after transfer. b: Cell layer 5 hours after transfer. Scale bar, 1 µm. Printed with permission from European Cells and Materials (ecmjournal.org).

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