Cell biology

A 5-step guide to fabricate and characterize cell sheets using Nunc Dishes with UpCell Surface

Introduction

A cell sheet comprises uniformly distributed confluent cells with preserved cell–cell junctions and extracellular matrix (ECM). Usage of scaffold-free cell sheets has emerged as a promising technique in tissue engineering research. Temperature-responsive surface is an effective tool to create and harvest intact cell sheets. Here we describe a simple protocol (Figure 1) for fabricating and harvesting single sheets as well as stacked sheets of human mesenchymal stromal cells (hMSCs) using the Thermo Scientific™ Nunc™ UpCell™ Surface. We have characterized a single cell sheet to ensure maintenance of both cell viability and the native ECM network. This protocol can be easily extended to other cell types as well.

Protocol

1. **Grow:** Seed the cells on a Thermo Scientific™ Nunc™ Dish with UpCell™ Surface at a density where they are confluent in a desired time and incubate at 37°C in 5% CO_2 to produce an intact sheet. The incubation time and cell seeding number are dependent on the cell type. For this study, 0.1 x 10^6 Wharton's jelly-derived MSCs (WJMSCs) were grown for 6 days on a 35 mm Nunc Dish with UpCell Surface coated with 20 µg/mL vitronectin using serum-free medium (Figure 2).

Confluent sheet not formed?

- Use cells in early passage (2–6) to produce a consistent cell sheet.
- Standardize the cell seeding number and/or incubation time.
- Add L-ascorbic acid (20–80 µg/mL) to the growth medium.

Notes

- Over- or under-seeding may result in uneven cell sheet formation.
- Ensure an even distribution of cells on the surface. Uneven seeding might lead to clumping.
- Feed the cells with fresh medium every other day to ensure proper nourishment of cells.

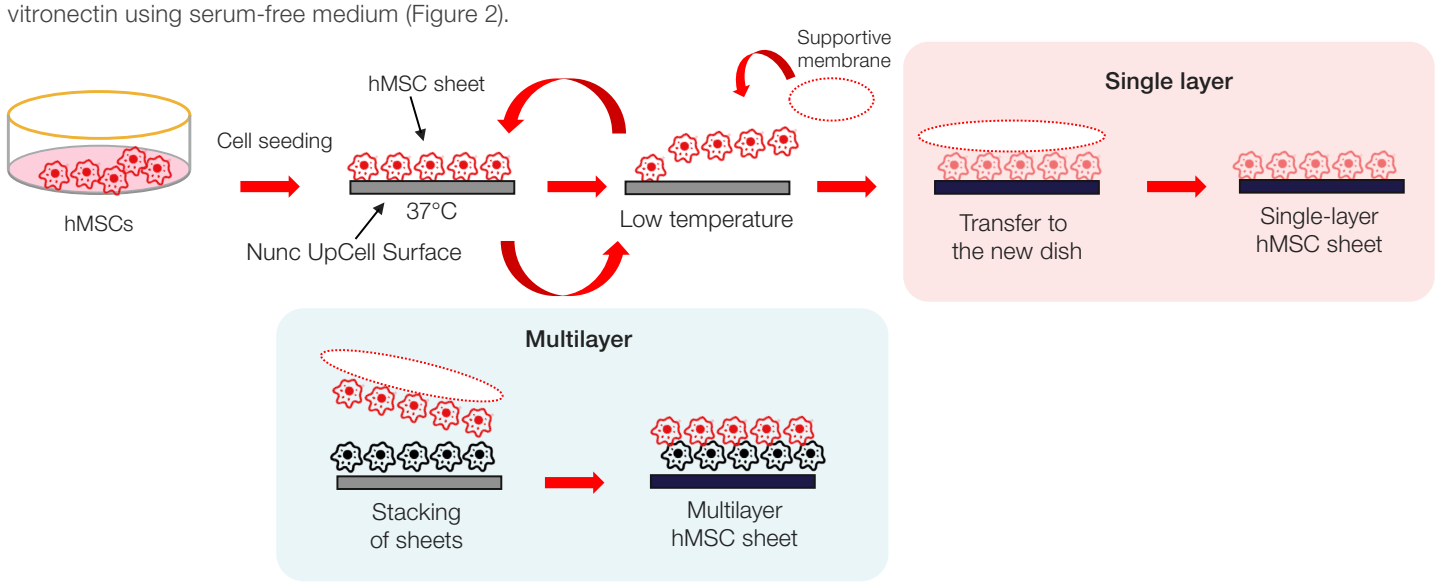

Figure 1. The process of generating and stacking hMSC sheets.

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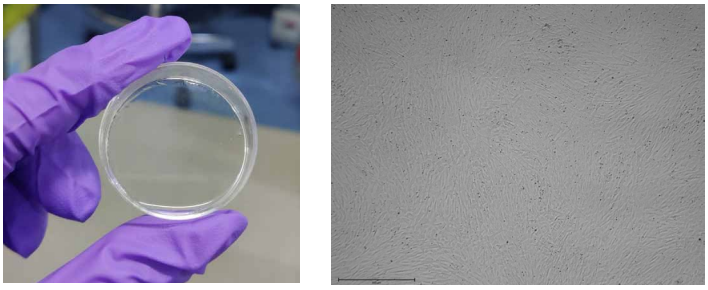

Figure 2. Growing the cells. (A) Confluent cell sheet of WJMSCs on 35 mm Nunc Dish with UpCell Surface. **(B)** Phase contrast image of confluent sheet.

2. **Overlay**: Once the cells are confluent, gently aspirate the medium and make a scratch around the edges of the confluent sheet using a 10 µL tip. Incubate the dish at 20–22°C to allow the cells to detach spontaneously. Normally, a cell sheet can be detached within 15–45 minutes. For this study, WJMSCs were detached in 20 minutes (Figure 3). Place the supportive membrane on the detached cell sheet using forceps. Because of its hydrophilicity, the membrane will quickly stick to cells while inhibiting cell sheet shrinkage and wrinkling.

Supportive membrane not sticking to the surface?

- Leave some medium (e.g., 200 µL for a 35 mm dish) behind in the dish to ease the overlaying of membrane.
- Make sure there are no trapped air bubbles while overlaying the membrane.

Notes

- The time until a cell sheet detaches spontaneously at 20–22°C depends on the cell type.
- To prevent folding of the cell sheet, place a micropipette tip on the other side of the supportive membrane while overlaying.

A B

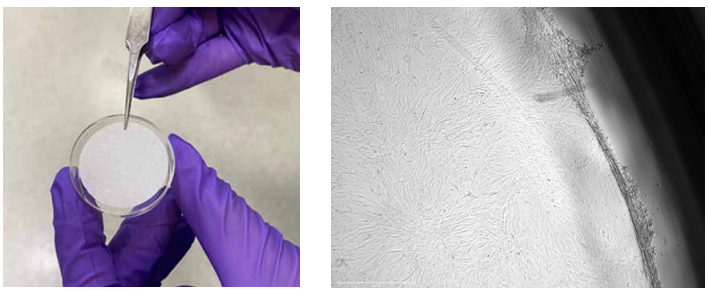

Figure 3. Overlay of the membrane. (A) Overlaying the supportive membrane onto the confluent sheet. **(B)** Microscopic image of the detachment of cells after temperature reduction.

3. **Recover:** Once the cells have detached from the surface, gently peel off the supporting membrane using forceps (Figure 4). The cell sheet attached to this membrane will also be harvested.

Is your cell sheet not recovered? Do you have a broken sheet?

- Remove the leftover medium from the dish to ensure proper peeling.
- Be very gentle while peeling off the supportive membrane.
- Incubate the dish at 22°C for longer to ensure sufficient time for the cell sheet to adhere to the supportive membrane.

A B

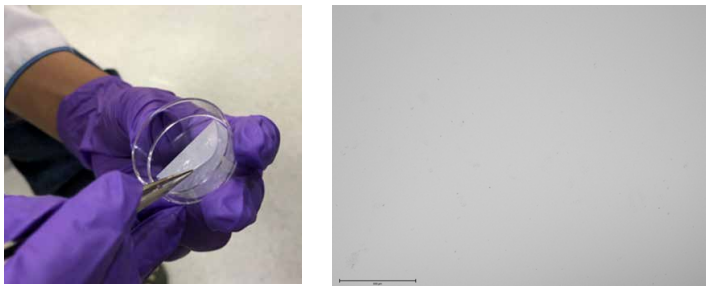

Figure 4. Recovery of the cell sheet. (A) Peeling off the supportive membrane with the attached cell sheet. **(B)** Microscopic image of Nunc Dish with UpCell Surface after complete recovery of the cell sheet (scale bar: 650 µm).

4. **Transfer and reattach:** Transfer the peeled supportive membrane with the cell sheet to the host surface and leave it at 37°C until the supportive membrane partially dries (Figure 5). This process usually takes 15–30 minutes. Here the WJMSC sheet attached to the membrane was incubated for 30 minutes at 37°C. Once partially dried, take the host surface out of the incubator, put a few drops of medium on top of the membrane, and gently peel it off, leaving the sheet of cells on the host surface. Since the ECM remains intact with the cell sheet, it helps in reattachment with the host surface.

Are you experiencing improper transfer and reattachment?

- Give sufficient time for cells to attach to the host surface.
- Do not peel off the membrane when it is still wet; give it some time to partially dry.

Note

- Leaving the supporting membrane for too long in the incubator will lead to drying and eventual cell death.

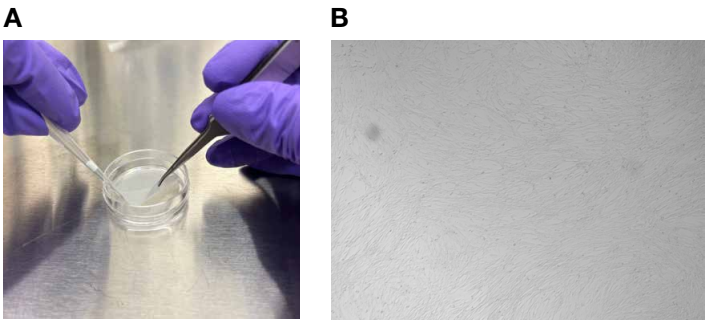

Figure 5. Transfer and reattachment of the cell sheet to the host surface. (A) Transferring the supportive membrane with the attached cell sheet onto the host surface. **(B)** Microscopic view of the transferred WJMSC sheet on the host surface.

5. **Characterize:** Characterization of the cell sheet can be done to ensure the formed sheet is live and the ECM markers are intact. For this study, the reattached cell sheet was stained with Invitrogen™ LIVE/DEAD™ stain and various ECM markers (Figure 6).

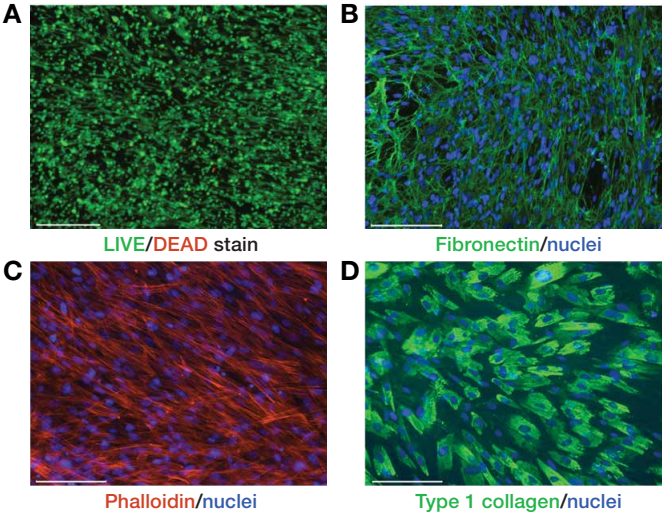

Figure 6. Characterization of the reattached cell sheet. **(A)** A LIVE/DEAD stain assay was used to view live cells (green) and dead cells (red). Immunofluorescent staining shows **(B)** cell nuclei (blue), fibronectin (green), **(C)** phalloidin (red), and **(D)** type 1 collagen (green). Images were taken using a 20x objective.

Stacking of cell sheets

A single cell sheet can be too thin for certain applications, so to generate thicker cell sheets, they are often stacked layer by layer to enhance their application in tissue engineering research. For generating multiple sheets, follow step 1 to grow the cells in multiple dishes. Follow step 2 to overlay the supportive membrane on the first sheet, followed by placement of the supportive membrane, holding the first cell sheet onto the second cell sheet and incubating at 20–22°C for 5 minutes to promote more stable adhesion between the two cell sheets. These two sheets can then be shifted to the third sheet and so on. Repeating this stacking process results in a multilayer construct.

To observe different layers, hMSC sheets were pre-labeled with Invitrogen™ CellTracker™ Blue, Green, and Red dyes, respectively (Figure 7). The cross-sectional images show the monolayer, double layer, and stack of multiple layers (Figure 8).

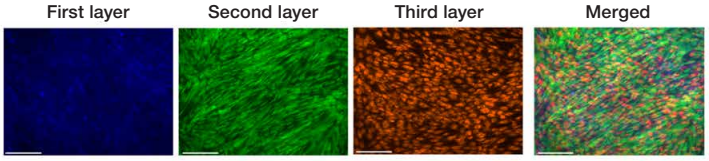

Figure 7. Visualization of cell layers. Pre-labeled cell sheets with Invitrogen™ CellTracker™ Blue, Green, and Red dyes were imaged using an Invitrogen™ EVOS™ M7000 Imaging System with a 10x objective.

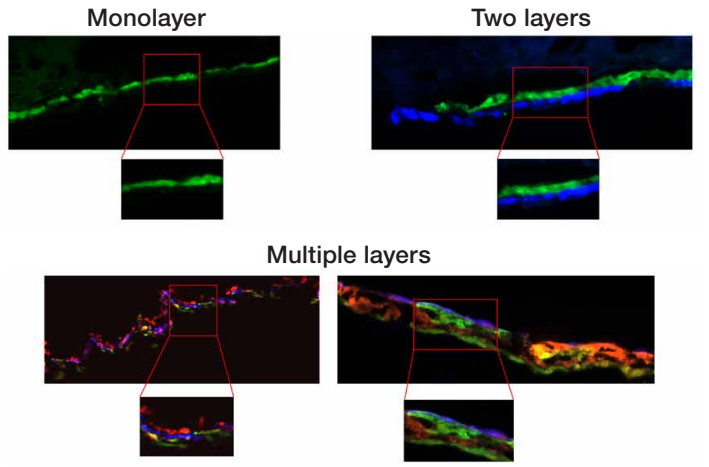

Figure 8. Cross-sectional views of cell layers. Pre-stained stacked sheets fixed using 4% paraformaldehyde were embedded in Tissue-Tek™ O.C.T. Compound (Sakura) and sagittally sliced in 10 µm sections using a cryotome (Leica Microsystems) and visualized using an EVOS M7000 Imaging System. Cross-sectional fluorescence images of the monolayer, double-layer, and multiple-layer cell sheets are shown.

Summary

Here in our 5-step guide, we have outlined a protocol for the fabrication of an MSC sheet using Nunc UpCell Surface, providing ways to obtain an intact sheet and techniques to avoid conditions which may lead to poor sheet quality.

This guide shows the generation, transfer, and stacking of viable cell sheets with preserved ECM markers and the creation of numerous different types of multilayer cell models, for research applications.

Ordering information

Product	Cat. No.
Nunc Dishes with UpCell Surface	174904
Vitronectin (VTN-N) Recombinant Human Protein, Truncated	A14700
StemPro MSC SFM XenoFree	A1067501
Fibronectin Polyclonal Antibody	PA5-29578
Collagen I Monoclonal Antibody (COL-1)	MA1-26771
Rhodamine Phalloidin	R415
NucBlue Live ReadyProbes Reagent (Hoechst 33342)	R37605
LIVE/DEAD Viability/Cytotoxicity Kit, for mammalian cells	L3224
CellTracker Red CMTPX Dye	C34552
CellTracker Green CMFDA Dye	C7025
CellTracker Blue Dye	C2110

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