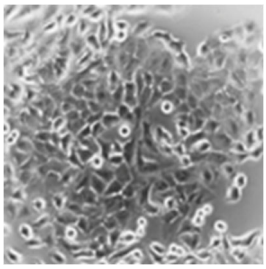
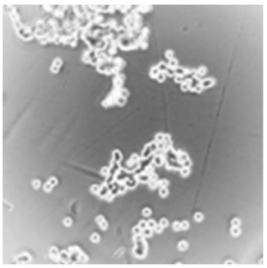
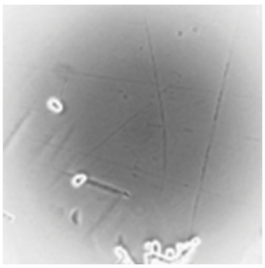
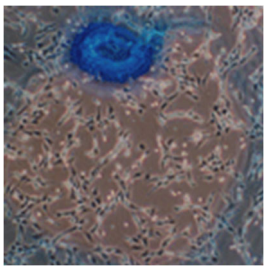
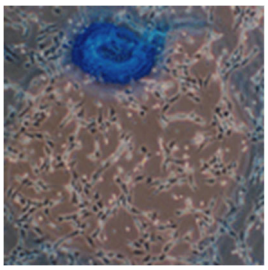
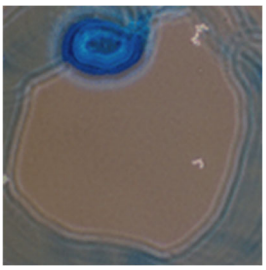
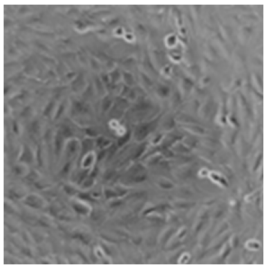
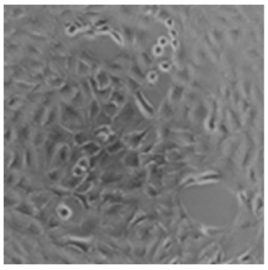
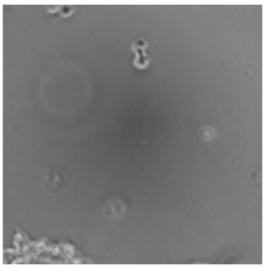
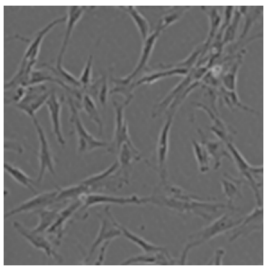
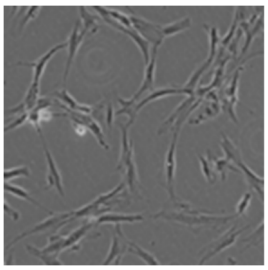
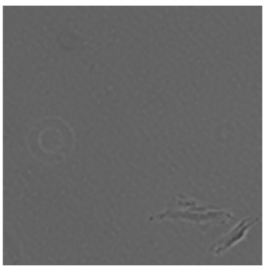
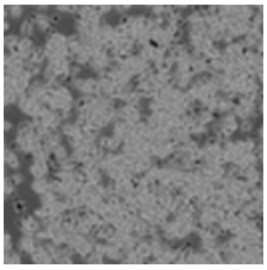
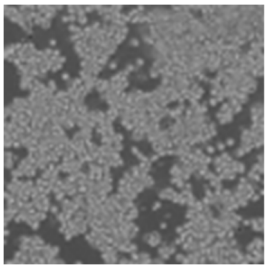
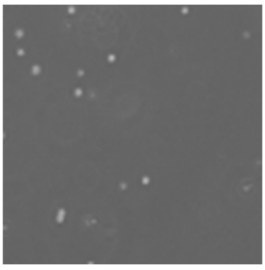
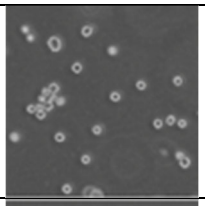
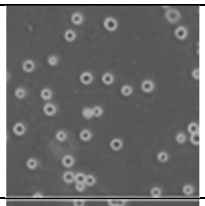
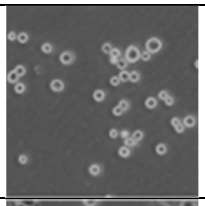
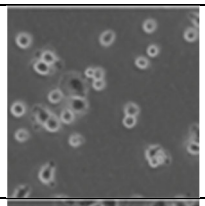
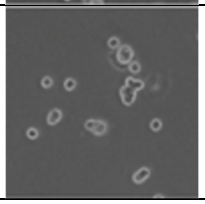
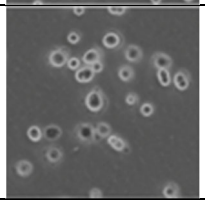
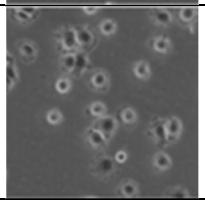
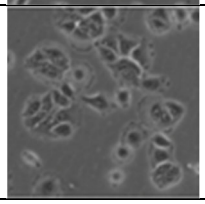
Application Data

1. Detachment of cells by control of temperature.
2. Comparison of re-attachment kinetics for harvested A-549 cells between UpCell®/RepCell® (UpCell® with Grid) and trypsin.
3. Comparison of harvested extra cellular matrix between UpCell®/RepCell® (UpCell® with Grid) and dispase (E-cadherin).
4. Comparison of flow cytometry application between UpCell®/RepCell® (UpCell® with Grid) and trypsin.
5. Comparison of detachment of macrophage between RepCell® and trypsin.
6. Comparison of re-attachment of macrophage between UpCell®/RepCell® and trypsin.
7. Protocol for macrophage collection in single cell suspension
8. Protocol for dendritic cell collection in single cell suspension.
9. Protocol of NIH/3T3 cell-sheet transfer using membranes for UpCell® 3.5cm.
10. Application data for mono-layered cell-sheet.
11. Application data for multiple layered cell-sheet (3D tissue constructs).

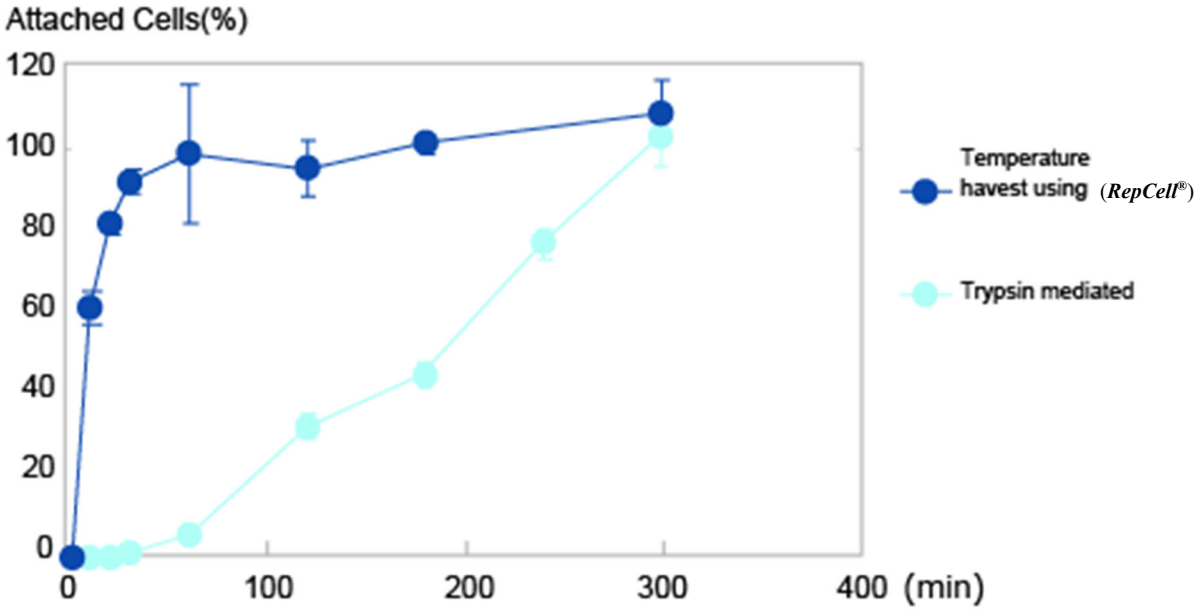
1. Detachment of cells by control of temperature.

	Normal culture @ 37 degrees celsius	30 min post-cool @ 20 degrees celsius	Removal after slight agitation
A549			
Swiss 3T3			
NRK			
Mesenchymal stem cells			
Peritoneal macrophage (mice)			

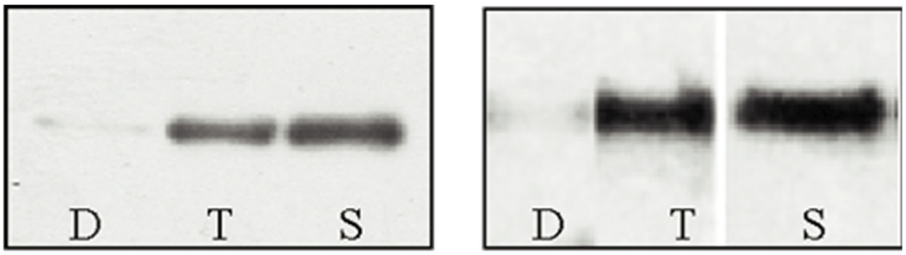
2. **Comparison of re-attachment kinetics for harvested A-549 cells between UpCell®/RepCell® (UpCell® with Grid) and trypsin.**

	10min	60min	120min	300min
Trypsin mediated				
Temperature harvest				

Comparison of re-attchment quantitative kinetics for harvested A-549 cells
Trypsin mediated vs temperature harvest.



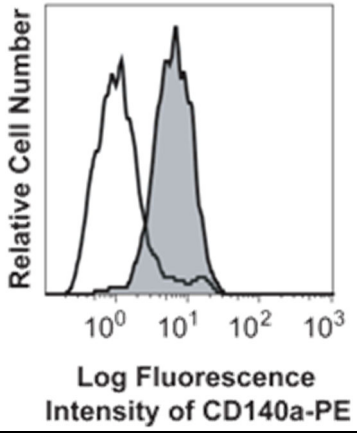
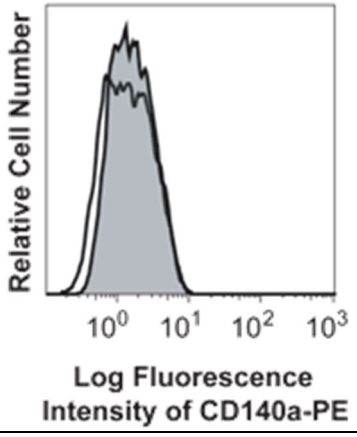
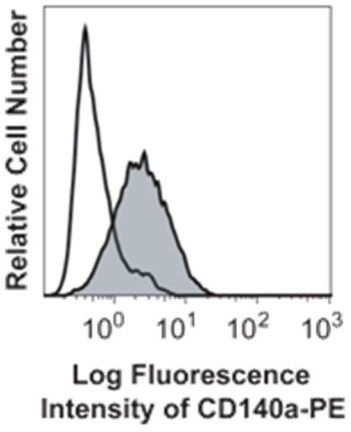
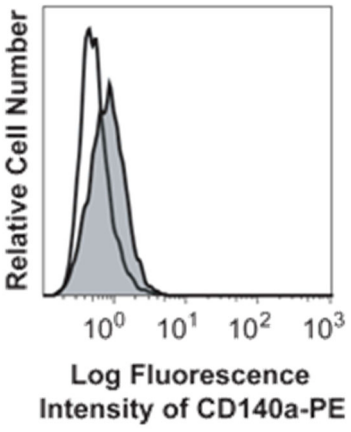
3. Comparison of harvested extra cellular matrix between UpCell®/RepCell® (UpCell® with Grid) and dispase (E-cadherin).



Human cornea epithlium　　Human keratinocytes

D：Dispase treatment T：Temperature harvest by RepCell® S：Physical scraping

4. Comparison of flow cytometry application between UpCell®/RepCell® (UpCell® with Grid) and trypsin.

	RepCell®	Trypsin
Bone marrow cells (human)		
Preadipocyte (human)		

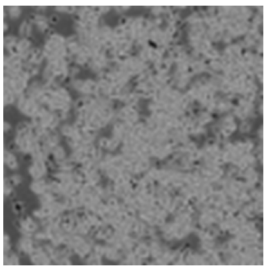
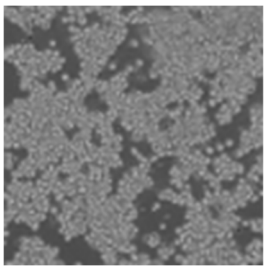
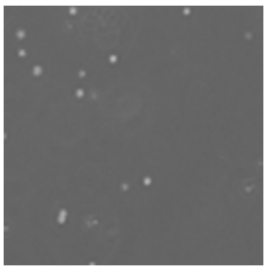
Methodologies

1. Seed 6.8 x 103/cm2 cells onto RepCell® and tissue culture treated dish.
2. Cells are harvested by temperature reduction for RepCell® (20℃, 30min) and trypsin (37℃, 3min).
3. Incubate detached cells with PE antibody for 1 hours at 4℃, followed by analysis with FC500 (Beckman Coulter).

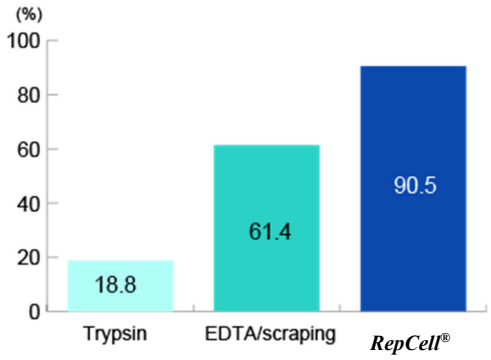
Antibody used

- D140a(PDGFRα)-PE:Pharmingen,556002,αR1, Ab1μg/2.5x105cells/0.2mL 1%FBS-PBS,
- Mouse IgG2a-PE(Isotype control):Pharmingen, 5592529, MPC-11, Ab 1μg/2.5x105 cells/0.2mL 1%FBS-PBS,

5. Comparison of detachment of macrophage between RepCell® and trypsin.

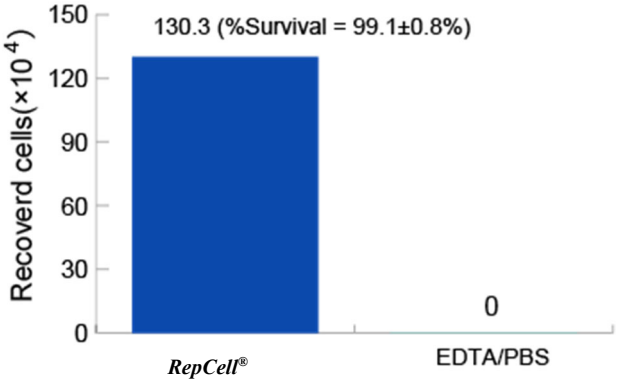
	Normal culture @ 37 degrees celsius	30 min post-cool @ 20 degrees celsius	Removal after slight agitation
Peritoneal macrophage (mouse)			

Recovery ratio of peritoneal macrophages (mouse)



1. Seed 5x106 of peritoneal macrophages (mouse) on 1 (one) RepCell® and 2 (two) tissue culture treated 6cm dish respectively.
2. Incubate for 2 hours, and wash off anchorage-independent cells by PBS.
3. Further culture for 2 days. Replace medium with PBS. Detach cell by temperature (on ice, 5min) for RepCell®, 2.5ml trypsin and EDTA/scraping for tissue culture dishes, followed by measurement.

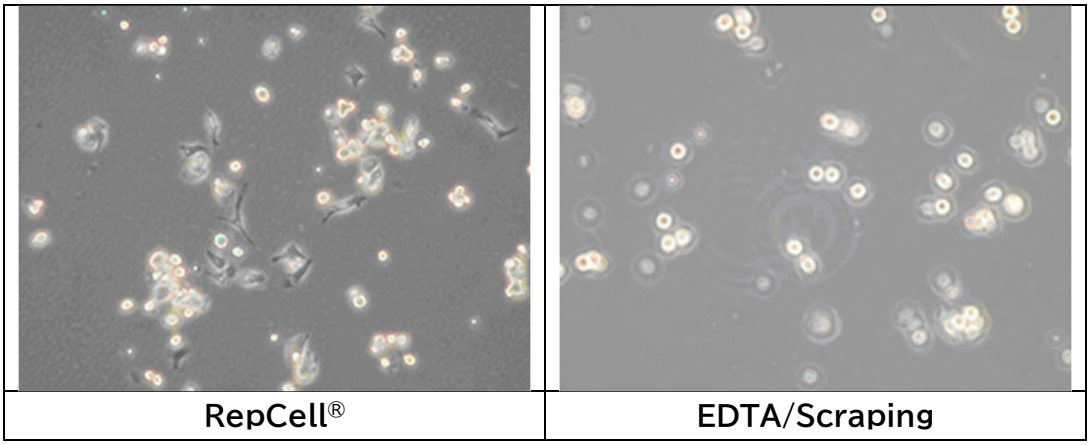
Recovery ratio of macrophage (human)



1. Seed 5x106 of HL-60 onto RepCell® and tissue treated cell culture dish (6cm). Add 100nm PMA (Phorbol 12 Myristate 130 Acetate) to induce cells to macrophages.
2. Culture for 5 days and replace medium with PBS. Detach cells by temperature (25℃, 30min) for RepCell® and EDTA/PBS for tissue culture treated dish.

(Prof. Kobayashi, Toho Univ. Biosci.)

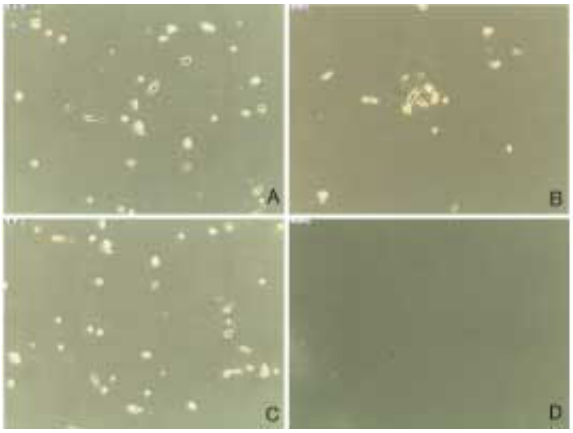
6. **Comparison of re-attachment of macrophage between UpCell®/RepCell® and trypsin.**

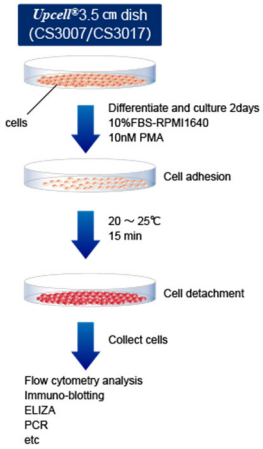


RepCell®	EDTA/Scraping

1. Seed 5x105 of peritoneal macrophages (mouse) onto RepCell® and tissue culture treated dish (6cm).
2. Incubate for 2 hours, followed by wash off of anchorage independent cells with PBS.
3. Further culture for 2 days. Replace medium with PBS. Detach cells with temperature (on ice, 5min) for RepCell® and PBS+EDTA.
4. Re-seed detached cells onto tissue culture dishes respectively.
5. Observe cells after 24 hours of incubation.

7. Protocol for macrophage collection in single cell suspension





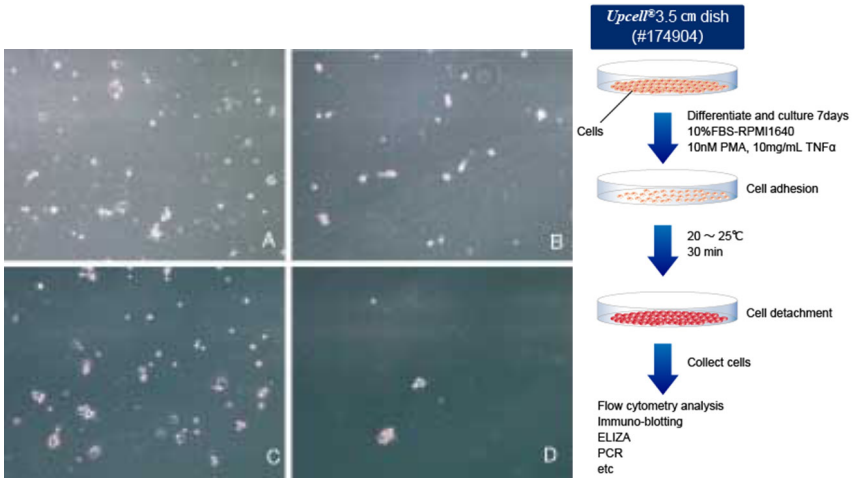
Cell culture conditions

1. Cell type : HL-60, JCRB0085/ATCC CCL-240
2. Cell density : 1.0 x 105 cells/UpCell® 3.5cm
3. Culture period : 2 days after differentiation induction
4. Culture medium : 10%FBS-RPMI 1640
   ∗ Cell culture condition may vary by cell type.
   (JCRB: Japanese Collection of Research Bioresources ATCC: American Type Culture Collection)

Methodology

1. Seed cells in low density so that cells do not stick to each other (A,B).
2. Leave UpCell® at room temperature (20-25℃) for 15 minutes.
3. Slight agitation with pipet will prompt cell detachment (D).
4. Cells do not come off for control dish (C).

8. Protocol for dendritic cell collection in single cell suspension.



Cell culture conditions

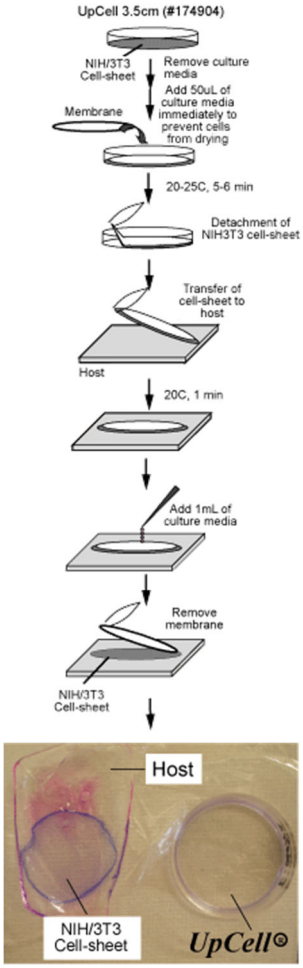
1. Cell type: KG-1, JCRB9051/ATCC CCL-246
2. Cell density: 2.0 x 105 cells/UpCell® 3.5cm
3. Culture period: 7 days after differentiation induction
4. Culture medium: 10%FBS-RPMI 1640, 10nM PMA, 10ng/mL TNFalfa*
   Cell culture condition may vary by cell type.

(JCRB: Japanese Collection of Research Bioresources ATCC: American Type Culture Collection)

Methodology

1. Seed cells in low density so that cells do not stick to each other (A,B).
2. Leave UpCell® at room temperature (20-25℃) for 30 minutes.
3. Slight agitation with pipet will prompt cell detachment (D).
4. Cells do not come off for control dish (C).

9. Protocol of NIH/3T3 cell-sheet transfer using membranes for UpCell® 3.5cm.



Cell culture condition

1. Cell type: NIH/3T3, ATCC CCL-1658
2. Cell density: 5.0 x 104 cells/UpCell® 3.5cm
3. Culture period: 7 days (over confluent)
4. Media: 10% FBS-DMEM

   * Cell culture condition may vary by the cell type. (ATCC: American Type Culture Collection)

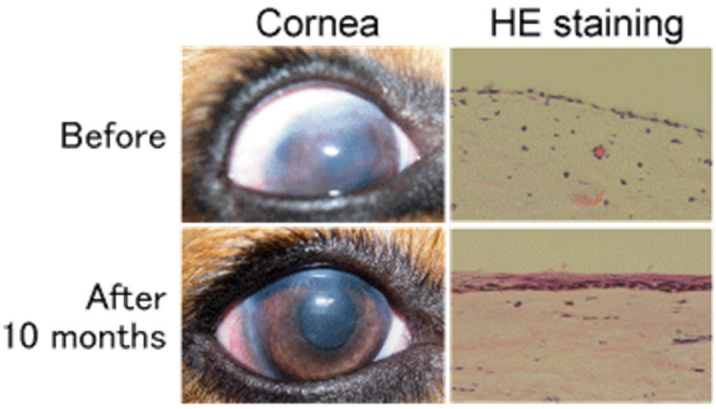
Methodology

1. Seed 5.0 x 104 cells of NIH/3T3 on UpCell® 3.5cm and culture for 7 days until cells become over- confluent. (Please note that some cells are difficult to culture beyond over confluency, in which case optimization of the culture condition is required.)
2. Take UpCell® out of incubator and remove culture media.
3. Immediately add 50uL of culture media to prevent cells from drying out.
4. Gently place membrane on the cell-sheet. Make sure there is no air bubble in between membrane and cell-sheet.

5. Leave UpCell® for 5-6 min at 20-25℃.
6. Gently peel membrane off from UpCell® using forceps.
7. Place cell-sheet with membrane to the host and wait 1 min. (In the left side of the diagram, the cell-sheet is transferred to sticky gel, mimicking the organ.)
8. Add 1mL of culture medium on the membrane to weaken the hygroscopicity of the membrane to release cell-sheet therefrom.
9. Carefully remove membrane only from cell-sheet using forceps. Make sure the cell-sheet is firmly attached to the host.
10. (Photo) NIH/3T3 cell-sheet has successfully been transferred to the gel. No cells failed to detach from UpCell®.

    * Cell culture/detachment conditions may vary by the cell types. Optimization may be necessary.

10. Application data for mono-layered cell-sheet.



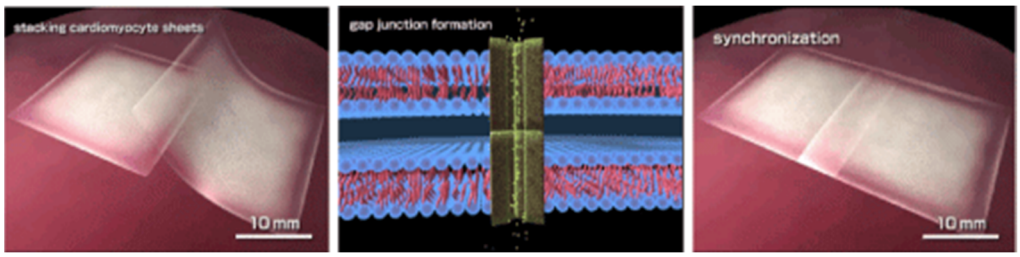
Autologous cornea epithelium cell-sheet has been transplanted to corneal limbal stem cell deficiency (LSCD) dog model. Reconstruction of the corneal surface has been achieved successfully. (Data kindly provided by Dr. Endoh (DVM), of Kunitachi Animal Hospital)

References on cornea epithelium reconstruction:

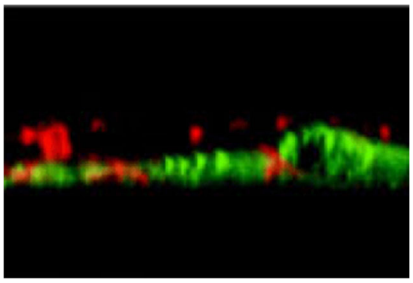
1. Hayashida, Y., Nishida, K., Yamato, M., Watanabe, K., Maeda, N., Watanabe, H., Kikuchi, A., Okano, T., and Tano, Y. (2005). Ocular surface reconstruction using autologous rabbit oral mucosal epithelial sheets fabricated ex vivo on a temperature-responsive culture surface. Investigative ophthalmology & visual science 46, 1632-1639.
2. Nishida, K., Yamato, M., Hayashida, Y., Watanabe, K., Yamamoto, K., Adachi, E., Nagai, S., Kikuchi, A., Maeda, N., Watanabe, H., Okano, T. and Tano, Y. (2004). Corneal reconstruction with tissue-engineered cell sheets composed of autologous oral mucosal epithelium. The New England journal of medicine 351, 1187-1196.
3. Nishida, K., Yamato, M., Hayashida, Y., Watanabe, K., Maeda, N., Watanabe, H., Yamamoto, K., Nagai, S., Kikuchi, A., Tano, Y. and Okano, T. (2004). Functional bioengineered corneal epithelial sheet grafts from corneal stem cells expanded ex vivo on a temperature-responsive cell culture surface. Transplantation 77, 379-385.

11. Application data for multiple layered cell-sheet (3D tissue constructs).

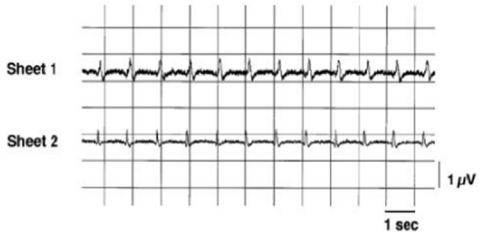
Construction of 3D tissue by layering cardiomyocyte cell-sheets (homogeneous cell-sheets)



- Place cardiomyocyte cell-sheet to another layer of cardiomyocyte cell-sheet.
- Since the cell-sheet retains viable cell surface proteins and channels, the vertical gap junction between cell-sheets can easily be established.



- Confocal image of 5 layered cardiomyocyte cell-sheets.



- Electric connectivity of the bi-layered cell-sheets has been established successfully.
- The vertical gap junction formation can be ahieved in 30 min.
- Synchronization was confirmed between the bi-layered cardiomyocyte cell-sheets.

References on 3D cardiomyocyte cell-sheets.

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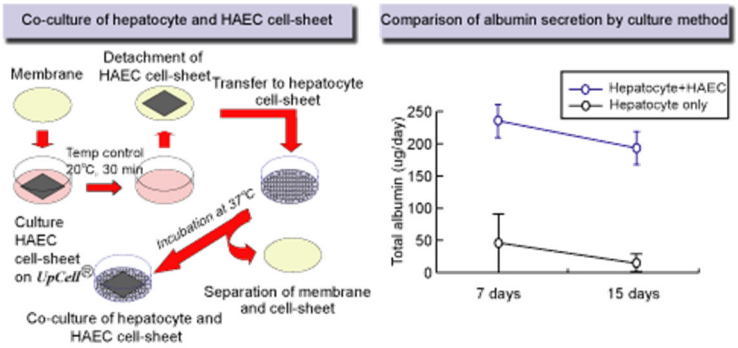
Construction of 3D tissue (heterogeneous cell-sheets)



- Hepatocyte is co-cultured together with HAEC (human aortic endothelial cell) cell-sheet to replicate ”in vivo”-like cell culture system.
- Co-cultured hepatocyte system maintained high level of albumin secretion (200ug/day) at day 15 whereas that of ”hepatocyte only ” system was very faint, suggesting that co-culture system resembles ”in vivo”-like culture. (Data kindly provided by Dr. Ohashi (MD, Ph.D), of Tokyo Women’s Medical Hospital)

References on co-culture system.

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