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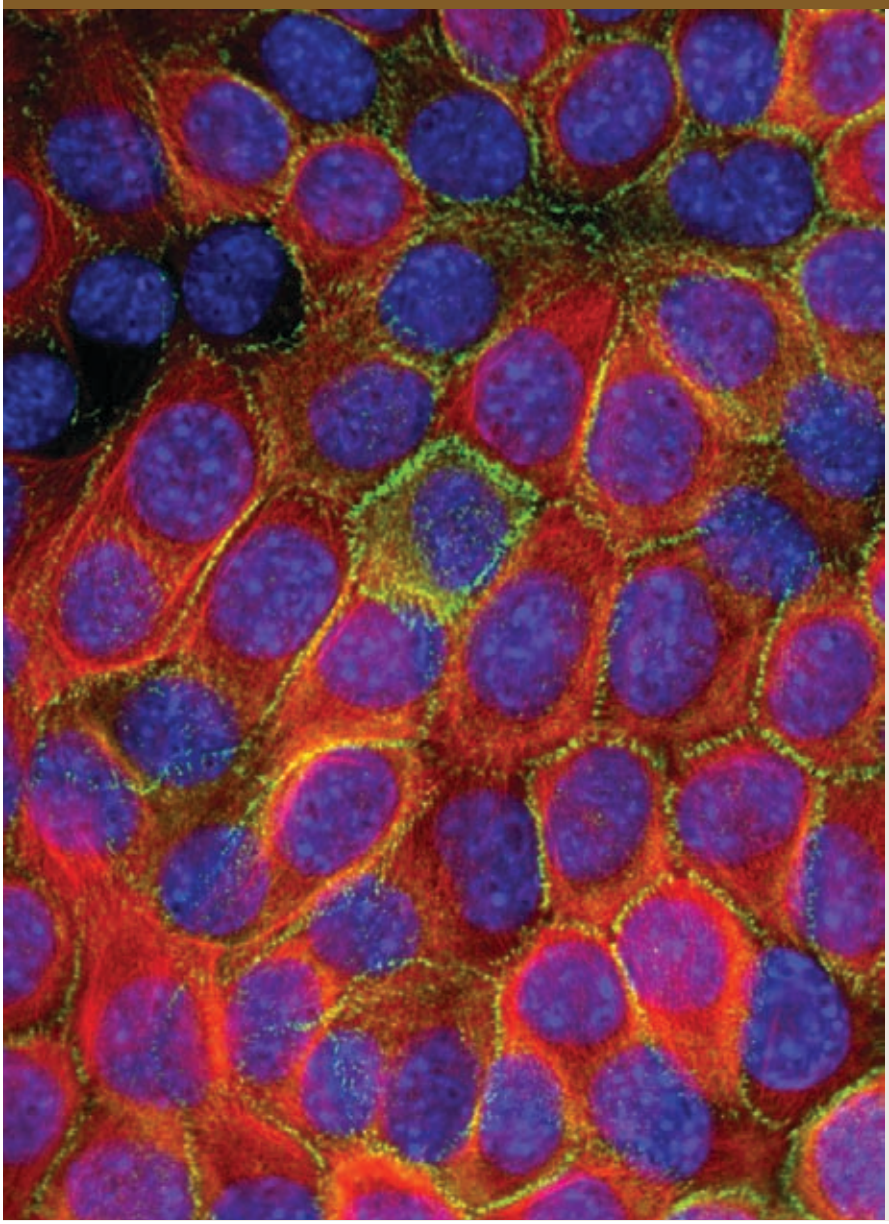

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In vitro modelling of Parkinson's disease using ReNcell VM human neural stem cell line

Erik A. Miljan, Ph.D., ReNeuron group plc

Abstract

The cellular mechanisms giving rise to Parkinson's disease remain largely unknown. Insights into these mechanisms can be drawn from investigating genetic mutations known to cause hereditary forms of the disease. PINK1 is one such gene associated with familial early onset Parkinson's disease. In this report, a novel cellular system involving PINK1 loss of gene function is described using the ReNcell VM cell line[1]. Interestingly, PINK1 knockdown in ReNcell VM cells did not affect dopaminergic neuron differentiation; however, long-term survival of the differentiated neuronal prodigy was markedly impacted by the loss of PINK1 function. Differentiated ReNcell VM neurons that contained the PINK1 knockdown displayed increased cell death via the mitochondrial apoptosis pathway when maintained in long-term culture. This reduction in viability was associated with increased oxidative stress and widespread mitochondrial dysfunction. To date, this is the first age-dependent neurodegenerative phenotype reported for any *in vitro* cell model of Parkinson's disease. By using ReNcell VM cells as a truly representative *in vitro* cellular model of the disease, these findings give fresh insights into the underlying cellular dysfunction that gives rise to Parkinsonian pathology.

Introduction

Parkinson's disease is the most common neurodegenerative motor disorder in the developed world. The mechanisms underlying the cause of this progressive and incurable disease remain unknown. The majority of Parkinson's disease cases are sporadic; however, inherited forms of Parkinson's disease have been linked to mutations in genes. Although hereditary forms present a much lower incidence than sporadic cases, the mutation in genes associated with hereditary forms of Parkinson's disease have provided insights into the pathogenesis of this disease. To date, a total of six genes have been strongly associated with the familial onset forms of Parkinson's disease; they include α-synuclein, parkin, DJ-1, PTEN-induced kinase 1 (PINK1), OMI/HTRA2, and LRRK2. The functions of these genes have implicated certain cellular process in the etiology of Parkinson's disease, namely impairment of the ubiquitin proteasome system, mitochondrial dysfunction, oxidative stress, and aberrant protein phosphorylation.

A novel cellular system involving PINK1 loss of gene function was generated using the ReNcell VM cell line to further understand the cellular mechanisms leading to Parkinson's disease[1]. It has been suggested that PINK1 is the second most causative gene associated with early onset inherited Parkinson's disease. PINK1 is a serine/threonine protein kinase that contains an N-terminal mitochondrial targeting motif. Physiochemical characterizations of PINK1 mutations associated with Parkinson's disease have shown that these mutations either obliterate protein kinase activity or give rise to premature mRNA decay. Together, these findings suggest that mutations in the PINK1 gene locus give rise to Parkinson's disease through loss of the PINK1 protein function. Indeed, knocking down PINK1 in ReNcell VM cells recapitulates *in vitro* the key features of Parkinson's disease—in particular, dysfunction and death in ageing human mid-brain neurons.

Methods

ReNcell VM cells are the ideal platform to model human neuronal disease in an *in vitro* paradigm. This cell line can be continuously grown in culture with a stable phenotype and genotype. In addition, ReNcell VM cells are well characterized and has the capacity to differentiate into human functional neuronal cells[2, 3]. In order to generate PINK1 loss-of-function in the ReNcell VM line, short hairpin RNA (shRNA) 19mer sequences targeted to PINK1 were designed and screened for their ability to disrupt wild-type PINK1 expression. Retroviral vectors were used to stably transform ReNcell VM cells with the shRNA constructs. Clonal cell lines were derived from the bulk transductions that contained the most effective PINK1-silencing shRNA construct. Differentiation of the shRNA transduced ReNcell VM clonal cell lines was carried out as previously described[2]. Cytotoxicity index assessment was carried out with an ArrayScan© HCS reader and analyzed using Multiparameter Cytotoxicity 1 BioApplication Software (Cellomics, Thermo Scientific) device and software.

Results and Discussion

Mouse transgenic models are typically used to study the effects of genetic mutations that give rise to disease states. To date, however, no Parkinsonian symptoms have been observed in such PINK1 loss-of-function mouse models. Although non-neuronal cell lines have been previously used to study the function of PINK1, they were cultured for relatively short periods of time and did not mimic the disease state. ReNcell VM was uniquely used in this application to provide a true, human neuronal PINK1-deficient *in vitro* model[1].

In the undifferentiated state, wild-type PINK1 expression in ReNcell VM cells are relatively low. However, an approximately 100-fold increase in PINK1 expression was observed upon differentiation. To induce PINK1 loss-of-function in ReNcell VM cells, retroviral vectors were used to stably transform PINK1 shRNA constructs into the cells. The various shRNA constructs were screened and found to result in a 10-90% decrease in PINK1 mRNA; the shRNA achieving the highest knockdown was used to derive clonal cell lines. Endogenous PINK1 knockdown was maintained even after differentiation as determined by qRT-PCR and Western blot.

The stably shRNA-transduced ReNcell VM clones were then studied in their differentiated state. Interestingly, PINK1 deficiency in ReNcell VM clones did not impact the neuronal differentiation capacity of these cells. There was no difference in the proportion of tyrosine hydroxylase (TH) positive cells compared with controls at early time points. Perhaps the most striking finding was the reduction in the long-term viability of neurons lacking PINK1 compared with controls; 43 days following induction of differentiation there was a significant decrease in viability and an increase in apoptotic cell death observed in the PINK1 knockdown clones. Furthermore, a steady increase in the cytotoxicity index (CI) was observed in PINK1 knockdown clones (Figure 1). The cell death observed in PINK1 knockdown clones was associated with increased production of basal free radicals and reduced levels of glutathione.

In addition, PINK1 knockdown cells displayed morphometric mitochondrial abnormalities. Transmission electron microscopy (TEM) revealed that aged PINK1 knockdown differentiated cells had abnormal swollen mitochondria (Figure 2). Further to mitochondrial abnormalities, large intracellular bodies comprised of multi-vesicular aggregates were observed solely in PINK1-deficient neurons. These aggregates were found to be lysosomal in nature and resembled autophagosomes. This represents the first demonstration that PINK1 may be implicated with lysosomal dysfunction. This is of great interest because lysosomes are thought to be crucial in the clearance of amyloid proteins, such as α-synuclein, implicated with Parkinson's disease pathology.

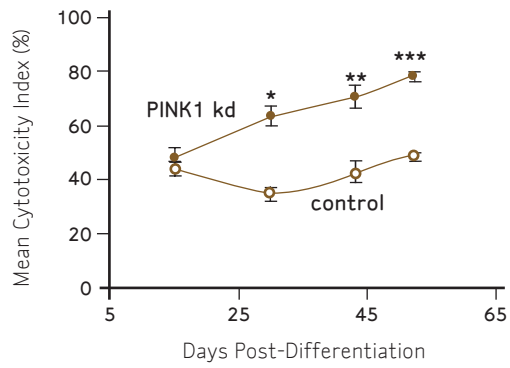

Figure 1. Graph showing an increase in the mean cytotoxicity index (CI) over time of ReNcell VM PINK1 knockdown clonal cells lines compared to normal ReNcell VM controls[1].

Conclusion

ReNcell cell lines provide the ideal platform to study human disease *in vitro*. Here, it was shown that ReNcell VM cells with PINK1 deficiency developed key Parkinsonian symptoms—namely age-dependent, progressive cellular dysfunction that ultimately lead to cell death. ReNcell neural stem cell lines are advantageous in that they provide a human model that can be continuously cultured, giving rise to a stable and consistent assay platform. Using the same methodology presented

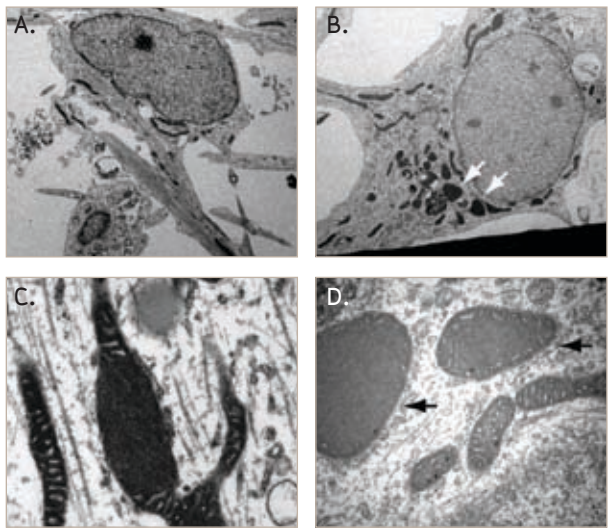

Figure 2. TEM images of control ReNcell VM (panel A) and aged ReNcell VM neurons lacking PINK1 (panels B-D). Abnormal mitochondria (arrowheads) are shown within ReNcell VM PINK1 knockdown cells (panel B, and at higher magnification in D). A higher proportion of mitochondria in PINK1 knockdown neurons appear swollen with disorganised christae (panels C, D).

herein, a number of neurodegenerative diseases with genetic implications could be modelled. In this context, ReNcell cell lines could be invaluable in the identification of new drug targets and high-throughput screening of potential drug candidates.

References

1. Wood-Kaczmar, A. *et al.* PINK1 is necessary for long term survival and mitochondrial function in human dopaminergic neurons. *PLoS ONE* **3**, e2455 (2008).
2. Donato, R. *et al.* Differential development of neuronal physiological responsiveness in two human neural stem cell lines. *BMC Neurosci* **8**, 36 (2007).
3. Hoffrogge, R. *et al.* 2-DE proteome analysis of a proliferating and differentiating human neuronal stem cell line (ReNcell VM). *Proteomics* **6**, 1833-47 (2006).

Related Products

Description	Catalogue No.
ReNcell VM Immortalized Cell Kit (cells + 500 mL of SCM005 maintenance media)	SCC010
ReNcell CX Immortalized Cell Kit (cells + 500 mL of SCM005 maintenance media)	SCC009
ReNcell NSC Maintenance Media, 500 mL	SCM005
ReNcell Neural Stem Cell Freezing Medium, 50 mL	SCM007
Human NSC Characterization Kit	SCR060
Human bFGF	GF003

Physiological relevance of ENStem-A human neural progenitors cultured in 3D polystyrene scaffolds

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Abstract

Three-dimensional (3D) cell cultures have potential to offer advantages in screening compound libraries for hits that can be developed as leads. Despite this potential, very few drug discovery laboratories use 3D cell cultures in their screening programs. One reason is that culturing cells in 3D for high throughput screening (HTS) in early drug discovery requires a platform compatible with contemporary instrumentation (e.g., 96-well format). Another reason is that the physiological relevance of 3D cultures is not well proven. We report herein the integration of 3D polystyrene scaffolds in multi-well standard plates. We cultured ENStem-A human neural progenitor cells in these plates and demonstrated the cultures to be more physiologically relevant using neural spheres as the *in vivo* surrogate.

Introduction

The long term goal in our Cellular Bioengineering Laboratory, as well as at SpatiumGen, is to develop and facilitate the commercialization of physiologically relevant, 3D cell-based assay platforms with applications in high throughput screening (HTS) of compound libraries. We have recently established a 3D cell culture platform by integrating (or chemically "welding") synthetic polymer (polystyrene) scaffolds into standard cell culture dishes and multi-well plates[1]. This technology can be used to feasibly modify traditional 2D cell culture vessels to 3D cell culture plates for use in contemporary HTS systems.

We cultured ENStem-A human neural progenitor cells in these 3D plates[2] and examined the cells' growth profiles, morphology, cell-matrix interactions, gene expression and voltage-gated calcium channel function[1]. We compared the 3D plate results to results from cells cultured on conventional 2D surfaces and cells in neural spheres (our *in vivo* surrogate). In comparison to 2D, cells in 3D plates better emulated cells from neural spheres, suggesting that 3D plates supported cells that were more physiologically relevant.

We are implementing this technology in a 96-well plate format. Eventually, we plan to convert a few standard 2D assays (currently in use in pharmaceutical and biotechnological drug discovery programs) to run in our 3D 96-well plates, with the goal of establishing the assays' robustness in terms of Z' factors. The results are expected

to facilitate the adoption of high throughput, 3D cell-based assays early in drug discovery. Below, we report some of our representative results.

Methods and Results

INTEGRATION OF POLYSTYRENE SCAFFOLDS WITH MULTI-WELL PLATES

Figure 2 shows the technique we employed to "weld" 3D polystyrene scaffolds into a standard plate. Generally, a viscous polymer solution was prepared by dissolving polystyrene in chloroform. Sieved ammonium bicarbonate particles with desirable sizes were added to the polymer solution and mixed thoroughly. The paste mixture was then cast into the wells of the plate with a single- or multi-channel pipette. After chloroform was completely evaporated, the plates were baked in an oven overnight. At temperatures above 36 °C, ammonium bicarbonate decomposes to ammonia, carbon dioxide, and water. This left pores, creating a porous polystyrene scaffold.

The resulting 3D plates offer the necessary spatial dimension (micro-scale pores) to allow formation of cellular aggregates and have minimal batch-to-batch variability as they are made with polystyrene as opposed natural materials. We are scaling-up the fabrication process with the use of automated liquid handlers which should yield precise well-to-well and plate-to-plate uniformity. Additionally, the use of polystyrene, a common polymer for standard 2D cell culture vessels, is expected to offer overall material cost advantages. Details of ENStem-A cell and neural sphere culture are published elsewhere[1].

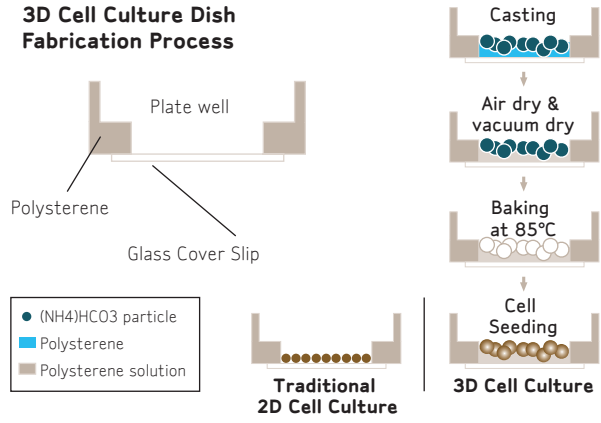

Figure 1. Fabrication process for integrating 3D polystyrene scaffolds into multi-well plates.

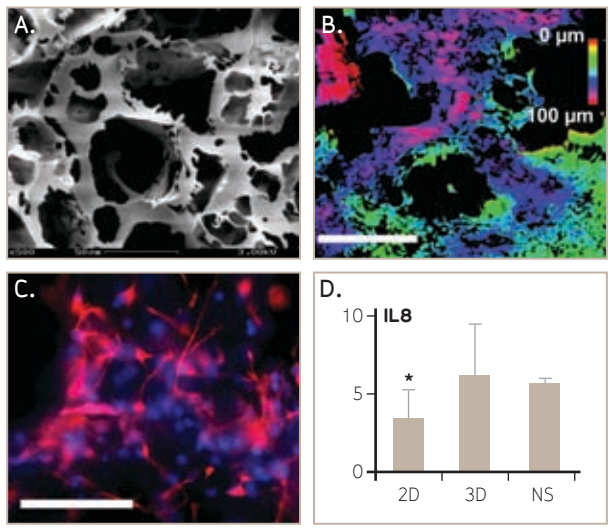

Figure 2. Human neural progenitor cells culture in 3D polystyrene scaffolds. (A) SEM micrograph shows the structure of the polystyrene scaffolds. (B) Confocal depth projection micrograph of NP cells in 3D scaffolds on day 2 after plating - 30 images taken in row by a z-scan were volume rendered. The color corresponds to the depth from the scaffold surface, with orange being closest to the surface and red being at 100 µm from the surface. Cells were stained with 5 µM Calcein-AM. (C) Immuno-fluorescence micrographs of NP cells stained with DAPI (blue) and Tuj (Red), after 14 days into differentiation in a 3D scaffold. (D) Gene expression level of IL-8 from cells in 2D, 3D and neural sphere (NS) cultures (4 biological replicates from each culture condition). * indicates $p < 0.05$.

HUMAN NEURAL PROGENITORS CULTURED IN 3D SCAFFOLDS ARE PHYSIOLOGICALLY MORE RELEVANT WHEN COMPARED TO THEIR COUNTERPARTS ON TRADITIONAL 2D SUBSTRATES

The porosity of the 3D scaffolds was above 85% with pore size precisely controlled by the salt particulate size in use (Figure 2A). The pores were interconnected with each other. With the aid of confocal laser scanning microscopy, a color depth projection image was created (Figure 2B) and confirmed that, after two days in culture, cells penetrated as deep as 100 µm from the surface toward the inside of the scaffolds. Immuno-staining of the neural marker Tuj showed that the cells in 3-D scaffolds differentiated into neurons after 14 days of culture in differentiation media (Figure 2C). To extend our study to the genomic level, we utilized the Human Whole Genome U133A 2.0 Plus GeneChip® Expression Analysis (Affymetrix, Santa Clara, CA) to compare the gene expression profiles between cultures on 2D planar surfaces, in 3-D scaffolds, and from neural spheres (*in vivo* surrogate). Our results indicated that the expression profile from 3D was much closer to the *in vivo* surrogate than the profile observed from 2D. For example, IL-8, a chemokine that belongs to a group of more than 50 relatively small

proteins, was equally up-regulated in both 3D and neural sphere cultures, and significantly less expressed in 2D cultures (Figure 2D). Growth profiles and voltage-gated calcium channel function were all significantly higher in 2D in comparison to 3D cultures (data not shown), consistent with the suggestion that cellular responses observed in 2D are probably exaggerations of the *in vivo* functionality[3]. In several published studies, physiologically important cellular functionalities have been observed in 3D cultures but not in 2D cultures[4, 5], bringing attention to the potential importance of including 3D assays in drug discovery programs[6].

Conclusion

Integrating 3D polymer scaffolds with standard cell culture vessels potentially offers a robust, ready-to-use, and highly compatible 3D cell-based assay platform for HTS assays in drug discovery programs. Our results showed that the 3D scaffolds supported growth, differentiation, and functionality of neural progenitor cells that closely emulated the *in vivo* surrogate (neural spheres), suggesting physiological relevance. Experiments are under way to demonstrate *in vivo* nerve tissue emulation.

References

1. Cheng K., Lai Y., Kisaalita W.S. (2008) *Biomaterials*. **29**: 2802-12.
2. Dhara S.K., Hasneen K., Machacek D.W., Boyd N.L., Rao R.R., Stice S.L. (2008). *Differentiation*. **76(5)**: 454-464.
3. Cukierman E., Pankov R., Stevens D.R., Yamada K.M. (2001). *Science*. **94**:1708-383.
4. Wolf K., Mazo I., Leung H., Engelke K., von Andrian U.H., Deryugina E.I., Strongin A.Y., Br_cker E.B., Friedl P. (2003). *Journal of Cell Biology*. **160**: 267–277.
5. Weaver V.M., Petersen O.W., Wang F., Larabell C.A., Briand P., Damsky C., Bissell M.J. (1997) *Journal of Cell Biology*. **137**: 231-245.
6. Mao C., Kisaalita W.S. (2004). *Biosensors and Bioelectronics*. **19**:1075-1088.

Related Products

Description	Catalogue No.
ENSTem-A Human Neural Progenitor Cells/Media Kit	SCR055
ENSTem-A Expansion Medium	SCM004
ENSTem-A Neuronal Differentiation Medium	SCM017
Human Neural Stem Cell Characterization Kit	SCR060

Creating your own 3D epidermal keratinocyte models using primary human keratinocytes, a 3D optimized culture medium, and Millicell inserts

Jim Johnson, CELLnTEC advanced cell systems, AG

Abstract

In the past, the preparation of three-dimensional (3D) epidermal *in vitro* models has been left primarily to specialized commercial providers, which supply established 3D models that must be used immediately upon delivery. Labs choosing to prepare their own models have often struggled with complicated protocols and the use of culture media designed for cell isolation and proliferation, rather than the specific demands of a 3D model requiring complete differentiation.

Human Epidermal Keratinocyte Progenitor Kit (cells and medium)

- **Two types**: HPEKP: Pooled donors
  HPEKS: Single donor
- **Two pack sizes**: HPEKP-05: >5 x 10^5 cells, plus medium
  HPEKS-05: 3 x >5 x 10^5 cells, plus medium

Each kit contains 500 mL of CnT-57 medium.

Millipore is proud to be the worldwide distributor for CELLnTEC products.

To fill this void, CELLnTEC developed a new 3D-optimized medium. Distributed by Millipore, this medium, along with CELLnTEC primary human keratinocytes and Millipore's Millicell inserts, can now be used to establish cost-effective 3D epidermal models. Together, these three products now enable researchers to routinely establish 3D epidermal models in their own lab, on their own schedule, using a proven protocol. In addition, this system uses completely defined culture media, giving researchers complete control of their experimental conditions and timing—all at a more attractive price.

Introduction

In recent years, a large body of research has shown that fresh primary cells more accurately model the *in vivo* situation than established cell lines. In addition, it has also been widely shown that 3D cell cultures, with their significantly improved differentiation and cell-cell contacts, also resemble the *in vivo* situation much more closely than 2D cultures in many situations. Accordingly, 3D cultures established using primary cells represent the most accurate method of modeling complex biological processes *in vitro*.

PROGENITOR CELL TARGETED (PCT) CULTURE MEDIA AND PRIMARY KERATINOCYTES

Primary keratinocytes (HPEK) isolated in PCT media have advantages not found in cells isolated using older media formulations. By mimicking the microenvironment of the adult stem cell niche, PCT media enhance the growth of progenitor cells, resulting in higher isolation efficiencies, and longer *in vitro* lifespan. PCT keratinocyte media are available in either fully defined (CnT-07), or low-BPE (CnT-57) formulations.

Primary HPEK keratinocytes (isolated in a PCT medium) are provided as a kit with a 500 mL bottle of culture medium. These cells are available both from pooled or single donor, in two different pack sizes. As a result of their excellent modeling and undifferentiated phenotype, these cells are ideal for establishing 3D *in vitro* models.

OPTIMIZED 3D KERATINOCYTE MEDIUM

The establishment of a 3D *in vitro* model places very specific demands on the cell culture medium. First, the medium must encourage cells to reach terminal differentiation as they stratify and establish the multiple layered structure. However, the medium must also maintain a population of proliferative

Figure 1. Millicell membrane inserts.

cells in the basal layer which will continue the supply of undifferentiated cells required by the model as it matures and differentiates.

These conflicting requirements are poorly addressed by most conventional media, which have been developed based solely on the need for isolation efficiency and proliferation, without consideration of these parallel requirements in a 3D model. To meet the specific and somewhat divergent needs of a 3D model, CELLnTEC has developed a new medium (CnT-02-3D), which has been designed with both the proliferation and

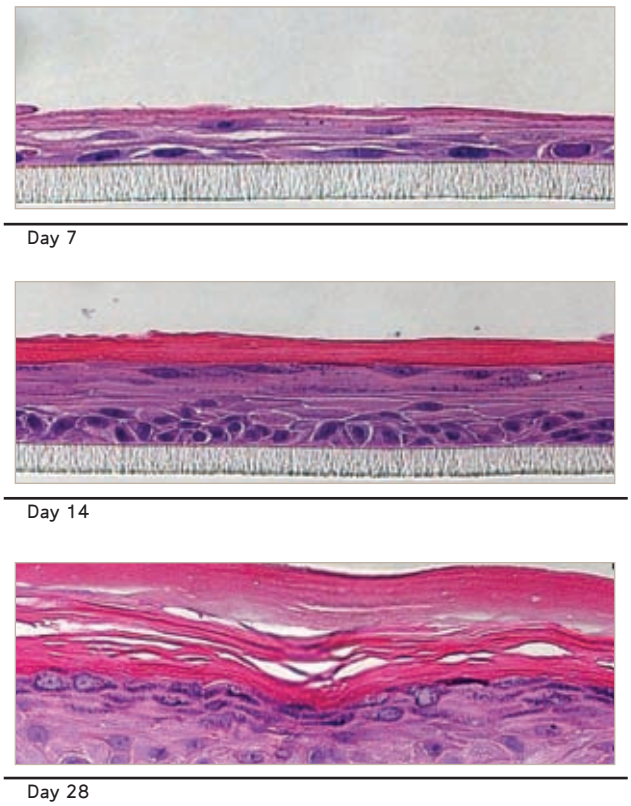

Figure 2. Human keratinocyte progenitors grown in epidermal keratinocyte 3D medium generate multiple strata of one epidermis after 28 days of culture.

differentiation needs in mind. In combination with primary human keratinocytes, this medium has been found to establish 3D epidermal models with accurate representation of the *in vivo* structure, and to maintain them for an extended period of at least 4 weeks. CnT-02-3D is available in both 100 mL (CnT-02-3D1) and 500 mL (CnT-02-3D5) pack sizes.

MILLIPORE MILLICELL INSERTS FOR 3D LIFTED CULTURE

3D epidermal models use a protocol in which the cells are lifted to grow at the air/liquid interface to encourage differentiation and stratification of the model. Millicell inserts from Millipore are a central element of this protocol. These feature a polycarbonate filter surface on which the cells are grown; culture medium diffuses through to reach the basal cells after the model is lifted to the air/liquid interface.

Millicell inserts enable excellent cell growth and attachment without the need for any additional coatings or matrices. Available in multiple configurations, we recommend the use of the 0.4 µm pore size for optimal adhesion and membrane diffusion. The Millicell inserts are provided sterile, individually blister-packaged, and ready to use.

Methods

To establish a 3D epidermal model, the following components are used:

- HPEK primary human keratinocytes (a kit including 500 mL PCT medium)
- CnT-02-3D medium for air/liquid interface culture
- Millicell inserts

See the following page for a listing of Catalogue Nos.

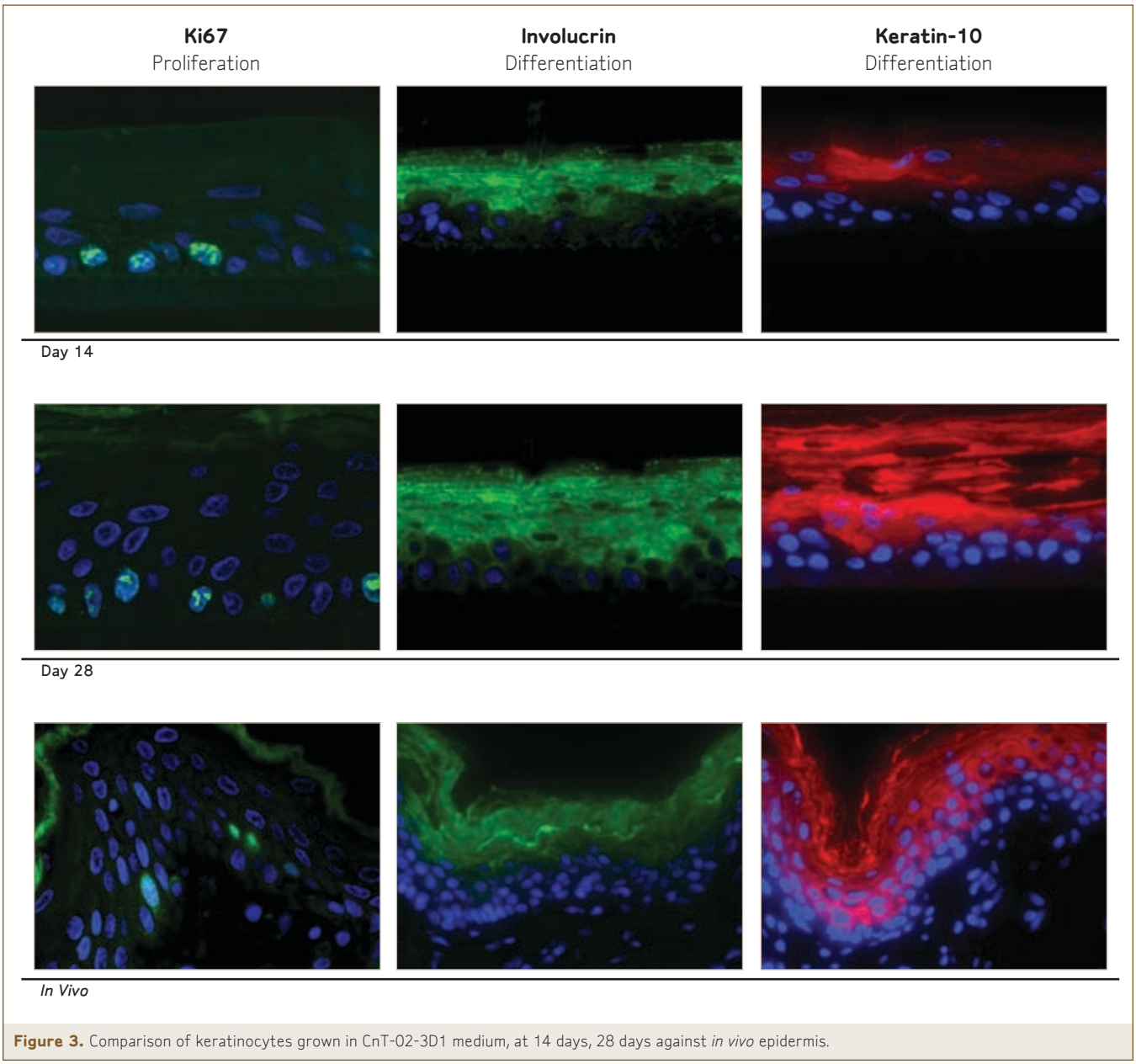

Figure 3. Comparison of keratinocytes grown in CnT-02-3D1 medium, at 14 days, 28 days against *in vivo* epidermis.

In summary, it progresses as follows:

1. Expand primary human epidermal keratinocyte progenitors (HPEKp.05) in CnT-57 medium (one cell kit can be expanded to provide enough cells for >100 inserts), then seed cells into Millicell PCF inserts (PIHP01250) and allow the cells to reach confluence (2-3 days).
2. Replace the CnT-57 medium with CnT-02-3D medium both under and over the inserts, and grow overnight to allow the cells to form intercellular adhesion structures.
3. Initiate the 3D culture by aspirating all the medium from inside the insert and replacing outside medium with fresh CnT-02-3D medium up to the level of the membrane (thereby "lifting" the culture).
4. The model will then continue to establish over the next 10-14 days, after which point it will be ready for experimentation. The model has been found to continue to proliferate for at least 28 days. For routine histological analysis, please see the recommended protocol.

A full protocol is available at **www.millipore.com** for establishment of the model.

Results and Discussion

As seen in the images on the previous page, the combination of CELLnTEC's HPEK primary keratinocytes, optimized CnT-02-3D medium, and Millicell inserts creates an excellent experimental system for 3D *in vitro* skin modeling. Cells grown in this way have been found to establish multiple strata of the epidermis (corneum, granulosum, spinosum, basale), and to express a range of adhesion and proliferation markers such as Ki67, involucrin, and keratin-10 (see images on previous page).

Using this system, normal epidermal structures can now be created routinely in your own lab, using a defined medium, and a protocol offering complete control of the experimental conditions, size and timing. These media and cell products are easily combined with the range of related antibiotic and enzyme products. Collectively, these synergistic product lines now represent a new level in cell culture performance, convenience, and cost-effectiveness.

Related Products – CELLnTEC Keratinocyte Media

Description	Size	Use	Catalogue No.
Epidermal Keratinocyte 3D Medium, defined	100 mL kit	3D Differentiation	CnT-02-3D1
Epidermal Keratinocyte 3D Medium, defined	500 mL kit	3D Differentiation	CnT-02-3D5
Epidermal Keratinocyte Medium, defined	500 mL kit	2D Differentiation	CnT-02
Epidermal Keratinocyte Medium, calcium free, defined	500 mL kit	2D Differentiation	CnT-02CF
PCT Epidermal Keratinocyte Medium, defined	500 mL kit	Isolation & Growth	CnT-07
PCT Epidermal Keratinocyte Medium, calcium free, defined	500 mL kit	Isolation & Growth	CnT-07CF
PCT Epidermal Keratinocyte Medium, low BPE	500 mL kit	Isolation & Growth	CnT-57
PCT Epidermal Keratinocyte Medium, calcium free, low BPE	500 mL kit	Isolation & Growth	CnT-57CF

Related Products – Keratinocytes, Millicell Inserts, and Reagents

Description	Size	Catalogue No.
Epidermal Keratinocyte Progenitors, pooled, human	1 x >5 x 10^5 cells and 500 mL media	HPEKP.05
Epidermal Keratinocyte Progenitors, pooled, human	3 x >5 x 10^5 cells and 500 mL media	HPEKP.15
Epidermal Keratinocyte Progenitors, single donor, human	1 x >5 x 10^5 cells and 500 mL media	HPEKS.05
Epidermal Keratinocyte Progenitors, single donor, human	3 x >5 x 10^5 cells and 500 mL media	HPEKS.15
Epidermal Keratinocyte Progenitors, C57BL/6, mouse	1 x >6.5 x 10^5 cells and 500 mL media	MPEK-BL6
Epidermal Keratinocyte Progenitors, Rosa, mouse	1 x >6.5 x 10^5 cells and 500 mL media	MPEK-ROSA
Epidermal Keratinocyte Progenitors, 129, mouse	1 x >6.5 x 10^5 cells and 500 mL media	MPEK-129
Antibiotic/Antimycotic Solution (100X)	100 mL	CnT-ABM
Antibiotic/Antimycotic Solution (200X, ready-to-use single aliquots)	10 x 2.5 mL	CnT-ABM10
Antibiotic/Antimycotic Solution (200X, ready-to-use single aliquots)	20 x 2.5 mL	CnT-ABM20
Neutral Protease (dispase), functionally tested	1 g	CnT-NPD-01
Millicell Single Well Inserts, PCF	0.4 µm pore size, 6 well, 50/pk	PIHP03050
Millicell Single Well Inserts, PCF	0.4 µm pore size, 24 well, 50/pk	PIHP01250

Tools for Neural Stem Cell Research

Millipore offers a comprehensive array of cells, kits, markers and cell culture products optimized specifically for neural stem cell research. Kits, media, and cells are highlighted in this newsletter. Please request our Neural Stem Cell Brochure (PB1090EN00) for detailed information, including our complete range of monoclonal and polyclonal antibodies for neural stem cell research.

Products available:

Species	Cell Lines (Derived = D; Isolated = I)	Cell Culture Products	Markers	Characterization Kits	Differentiation Kits
Human	✔ (D)	✔	✔	✔	✔
Mouse	✔ (I)	✔	✔	✔	✔
Rat	✔ (I)	✔	✔	✔	✔

Kits

	Catalogue No.	Description
Rodent Neuron Differentiation Kit	SCR035	Kit provides two neuronal inducers that, when added to a defined serum-free medium, allow for the preferential differentiation of rodent neural stem cells to a neuronal lineage. Other components of the kit include fixation buffer and two antibodies for the immunocytochemical characterization of the resulting neuron population.
Adult Rat Neural Stem Cell Expansion Kit	SCR034	These kits provide a multi-component system for the culture and analysis of neural stem cells and their differentiated progenies. These systems include primary neural stem cells, neural stem cell expansion medium, and a panel of antibodies for the immunocytochemical staining of neural stem/progenitor cells (nestin and Sox2) and differentiated neural phenotypes (Map2ab for neurons, GFAP for astrocytes and O1 for oligodendrocytes).
Mouse Cortical NSC Expansion Kit	SCR032	
Mouse Spinal Cord NSC Expansion Kit	SCR033	
Human Neural Stem Cell Characterization Kit	SCR060	Kit contains a panel of markers that are frequently used to identify neural stem cells/ progenitors. Includes nestin, Sox-2, Musashi, βIII-tubulin for neurons, GFAP for astrocytes and O1 for oligodendrocytes.
Embryonic Stem Cell Derived Neuron Integration and Characterization Kit	NS140	Kit allows for the localization, characterization, and analysis of ES cell-derived neuron integration into adult central nervous system (CNS) tissue. The kit includes antibodies for the identification of glutamate neurons (EAAC1), GABAergic neurons (GAD67), dopaminergic neurons (TH), and serotonergic neurons (serotonin). It also includes antibodies for the general identification of synapses (PSD-95 and synaptophysin) as well as for GABAergic synapses (GABA A receptor β-chain).
Neuron-Glial Cell Marker Sampler Kit	NS130	Kit contains antibodies for the identification of neurons (βIII-tubulin and Map2ab), astrocytes (GFAP), and oligodendrocytes (RIP).
Dopaminergic Neuron Integration and Characterization Kit	NS145	Kit includes antibodies for the identification of dopaminergic neurons in different stages of neurotransmitter synthesis and maintenance: dopa decarboxylase; tyrosine hydroxylase (TH); neurons (NeuN), astrocytes (GFAP) and markers for the identification of cholinergic (ChAT) and serotonergic (serotonin) neurons.

Media

	Catalogue No.	Description
Astrocyte Differentiation Medium	SCM010	Specially formulated medium optimized for the preferential differentiation of rodent neural stem cells to an astrocyte lineage. The medium has been extensively validated on mouse cortical and spinal cord neural stem cells and on rat hippocampal neural stem cells.
NDiff Neuro-2 Medium Supplement	SCM012	N2- and B27-like medium supplements for the serum-free culture of mouse ES cells into post-mitotic neurons and for the derivation, propagation, and maintenance of mouse NSCs.
NDiff Neuro-27 Medium Supplement	SCM013	
Mouse Neural Stem Cell Expansion Medium	SCM008	These expansion media are provided as two-component systems that are convenient and easy-to-use. Kits include the Neural Stem Cell Basal Medium and the necessary supplements, which allow for the growth and proliferation of mouse and rat neural stem cells.
Rat Neural Stem Cell Expansion Medium	SCM009	
Neural Stem Cell Basal Medium	SCM003	Defined serum-free, growth factor-free medium that has been optimized for the growth and *in vitro* differentiation of neural stem cells derived from rodents. When used in conjunction with bFGF or bFGF, EGF, and heparin, the basal medium allows for the proliferation of rat and mouse neural stem cells, respectively.
Neural Stem Cell Freezing Medium	SCM014	Qualified for use with mouse and rat neural stem cell lines cultured in serum-free conditions with Millipore's NSC Basal Medium. The optimized formulation enables consistent cryopreservation and high viability upon thawing and plating.
ENStem-A HNSC Expansion Medium	SCM004	Defined, serum-free, complete medium for convenient, reliable expansion of ENStem-A Human Neural Progenitors.
ENStem-A HNSC Neuronal Differentiation Medium	SCM017	Defined, serum-free medium for directed differentiation of ENSTem-A Human Neural Progenitors into neuronal populations.
ENStem-A HNSC Freezing Medium	SCM011	Defined, serum-free medium for freezing ENSTem-A Human Neural Progenitors.
ReNcell HNSC Maintenance Medium	SCM005	Defined, serum-free Medium for convenient, reliable culture of ReNCell VM and ReNcell CX Human Neural Progenitors.
ReNcell Freezing Medium for ReNcell Human Neural progenitors	SCM007	Defined, serum-free medium for freezing ReNcell Human Neural Progenitors.

Cells

	Catalogue No.	Description
ReNcell VM Human Neural Progenitor Cells, derived from ventral mesencephalon	SCC010	ReNcell VM and ReNcell CX cell lines are immortalized cells derived from human fetal neural tissue. These cells have been extensively validated and shown to differentiate into cells of all three major neuronal pathways (astrocytes, neurons, and oligodendrocytes). Cells are sold with 500 mL of specially optimized ReNcell maintenance media (SCM005) and freezing media (SCM007). (Media is also available separately.)
ReNcell CX Human Neural Progenitor Cells, derived from cortex	SCC009	
ENStem-A Human Neural Progenitor Cells, derived from hES Cells	SCR055	Generate normal, functional neurons from hES–derived neural progenitors. ENStem-A cells are derived from the human embryonic stem cell line, WA09. They have been validated and have been shown to readily differentiate into neurons and glia. They are sold with 500 mL of optimized ENStem-A Expansion Medium containing bFGF.
Millitrace™ Constitutive GFP Reporter Adult Rat Hippocampal NSC Kit	SCR080	Rodent neural stem cell (NSC) lines that express green fluorescent protein (GFP) constitutively. GFP expression in these stem cells allows researchers to easily monitor the behavior of specific populations of cells as they proliferate, migrate, and differentiate into various cell lineages, depending on developmental context.
Millitrace Constitutive GFP Reporter Mouse Cortical NSC Kit	SCR081	
Mouse Cortical Neural Stem Cells	SCR029	Ready-to-use, primary cells for a variety of research applications, including drug development, studies of neurotoxicity, neurogenesis, electrophysiology, neurotransmitter and receptor functions and CNS diseases and disorders. Each lot of primary cells has been validated for high expression of the appropriate markers, and, with respect to adult rat NSC, for their self-renewal and multi-lineage differentiation capacities.
Mouse Spinal Cord Neural Stem Cells	SCR031	
Adult Rat Hippocampal Neural Stem Cells	SCR022	
Rat Hippocampal Neurons	SCR010	
Rat Hippocampal Astrocytes	SCR008	
GFP Reporter Cell Lines for Human Neural Stem Cells	COMING SOON!	

Optimization of extracellular matrix proteins for *in vitro* cell culture systems

Christine Chen, Ph.D., Yiwen Jan, Ph.D., Vi Chu, Ph.D. and Robert Kovelman, Ph.D., Millipore Corporation, Temecula, CA

Abstract

Determining the optimal extracellular matrix (ECM) proteins and concentrations for cell culture can be a difficult, time-consuming process. In this study, we evaluated Millipore's ECM Cell Culture Optimization Array as a tool for identifying the ECM protein and concentration requirements for neural stem cell attachment. We also examined the ECM/integrin interaction by blocking collagen-1 or vitronectin receptors in cancer cells with various integrin antibodies.

Introduction

Extracellular matrix (ECM) proteins are produced intracellularly and are secreted into the surrounding cellular medium, actively regulating a diverse range of cell functions including adhesion, differentiation, proliferation, migration, invasion, and survival. ECM proteins are critical for the *in vitro* culture of many known cell types, including neural stem cells, and are key building blocks of the *in vivo* 3-D cellular environment. A primary use of ECM proteins in *in vitro* cell culture is to promote cellular adhesion, while maintaining viability and maximizing proliferation for downstream cell-based applications. In cases where optimal cell growth conditions are not well defined and adhesion protein requirements are unknown, the ideal ECM protein(s) and physiologically relevant concentration(s) must be determined. Only then can research proceed to examine cell functions such as integrin-mediated signaling and cell migration.

Millipore has designed a straightforward, plate-based assay to solve this problem. The ECM Cell Culture Optimization Array is pre-coated with four ECM proteins (human collagen I, mouse laminin, human fibronectin, and human vitronectin) in concentrations ranging from 0.1 to 20 µg/mL.

ECM proteins like these play an important role in stem cell research, including the understanding and development of a microenvironment that mimics the neural stem cell (NSC) niche and supports growth (2). In our experiments, both human and rat NSCs were used to profile the cells' preference to these common ECM proteins.

We also examined the integrin/ECM interaction in cancer cells, as the activation of the integrin signaling pathway in cancer cells through their interaction with the surrounding ECMs is thought to regulate the cell-cell interaction and further downstream activities, such as metastases and invasion.

Materials & Methods

CELL CULTURE

All materials were obtained from Millipore unless indicated otherwise. ReNcell VM human neural stem cells (isolated from ventral mesencephalon and immortalized) were cultured in ReNcell medium in the presence of 20 ng/mL FGF-2 and 20 ng/mL EGF. Primary rat neural stem cells were isolated from the hippocampus of adult Fisher 344 rats and cultured in Rat Neural Stem Cell Expansion Medium in the presence of 20 ng/mL FGF-2. Human malignant skin melanoma line A375 was cultured in DMEM supplement with 10% fetal bovine serum. Human lung carcinoma line A549 was cultured in DMEM/F12 supplement with 10% fetal bovine serum. All cells were maintained at 37 °C in a 5% CO_2 incubator.

CELL ADHESION ASSAY

The ECM Cell Culture Optimization Array was thawed at room temperature or 4 °C prior to use. After thawing, liquid was removed from wells and the plate was rinsed once with PBS. Each well was blocked with 100 µL of 0.5% BSA at room temperature for 1 hour before adding cells.

Cells were harvested with Accutase™ solutions when they reached 80 to 90% confluence. Cells were then washed twice with pre-warmed basal medium without serum and finally resuspended to 0.5-2 x 10^6 cells/mL. 100 µL of this cell suspension was added to each well and incubated for 1 to 2 hours at 37 °C in a 5% CO_2 chamber. After incubation, non-attached cells were washed out by rinsing the well gently 4 times with pre-warmed serum-free medium.

For colorimetric detection, each well was incubated with 100 µL of cell stain solution at room temperature for 10 minutes followed by washing 4 times with distilled water. Stained cells were retrieved with 100 µL cell extraction solution and quantified by spectrometer at 570 nm. For fluorometric detection, each well was stained with 50 µL of 1 µM calcein-AM solution at 37 °C for 15 minutes followed by 2-3 washes to remove excess dye. Stained cells were directly quantified by fluorometer at 514 nm.

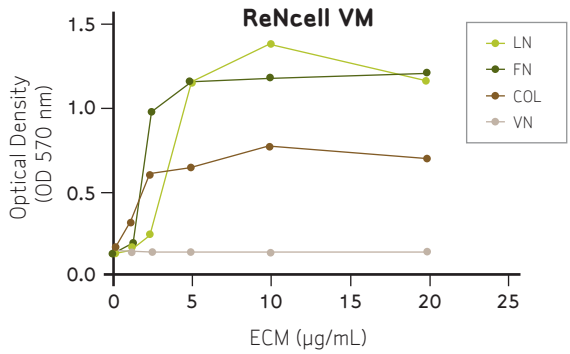

Figure 1. ReNcell VM adhesion profiling against ECMs. Human neural stem cells, ReNcell VM, were seeded on the ECM Cell Culture Optimization Array at 10^5 cells per well for 2 hours at 37 °C. Cell adhesion levels were measured by crystal violet staining and analyzed by spectrometer. Each data set represents three replicates.

In order to block the integrin/ECM interaction, 20 µg/mL of mouse anti-human integrin (α1, α2β1, αvβ3, or αvβ5) antibodies were incubated with either A-375 or A549 cell suspension at 37 °C for 1 hour prior plating onto an ECM-coated plate. Cell adhesion was evaluated as described above.

Experimental data were imported into GraphPad Prism® 4 to calculate the standard error and create graphic layout.

Results and Discussion

Both types of NSCs have preferentially adhered to laminin and fibronectin, demonstrating less attachment to vitronectin and none to collagen-1 (Figures 1 and 3). Cell morphology observations also supported the profiling result that the cells attached to laminin or fibronectin were flat with extensive spreading (Figures 2 and 4; cell morphology on fibronectin substrate (b) and laminin substrate (c)). Based on the graphic result, the optimal concentrations for human NSC attachment to fibronectin and laminin are 5 µg/mL and 10 µg/mL, respectively. The optimal concentrations for rat NSC attachment to fibronectin and laminin are 2.5 µg/mL and 10 µg/mL, respectively.

The above results were collected using the colorimetric method, which measures cell numbers by staining with crystal violet, a nucleic acid dye, and requires the attached cells to be fixed prior to staining. In order to measure living cell attachment using the fluorometric method, the ReNcell VM cells were stained with calcein-AM after attachment. Comparing data on Figure 1 and Figure 5 suggests that the two detection methods did not yield significant differences. Therefore, selection between the two methods should be based upon the desired outcome: colorimetric staining provides a fast and easy protocol, while fluorometric staining provides better sensitivity and allows subsequent analysis, such as immunofluoresent staining.

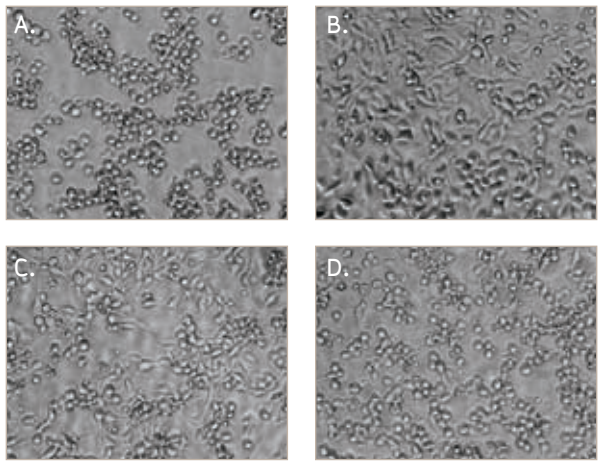

Figure 2. Brightfield Image of ReNcell VM on different ECM substrates. Cell morphology of ReNcell VM when attached to (A) collagen-1, (B) fibronectin, (C) laminin, and (D) vitronectin at 20 µg/mL concentration. Note that the attached cells showed flat morphology while unattached cells showed spherical morphology.

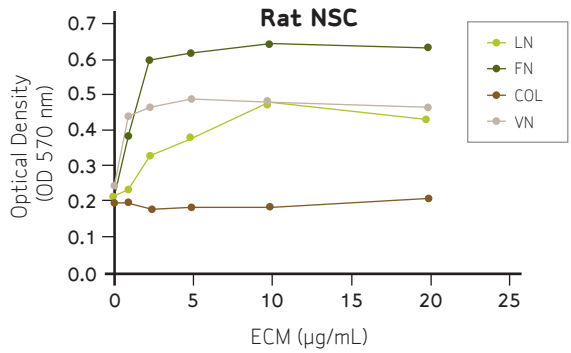

Figure 3. Rat NSC adhesion profiling against ECMs. Rat hippocampal neural stem cells were seeded on the ECM Cell Culture Optimization Array at $0.5x10^5$ cells per well for 2 hours at 37 °C. Cell adhesion levels were measured by crystal violet staining and analyzed by spectrometer. Each data set represents three replicates.

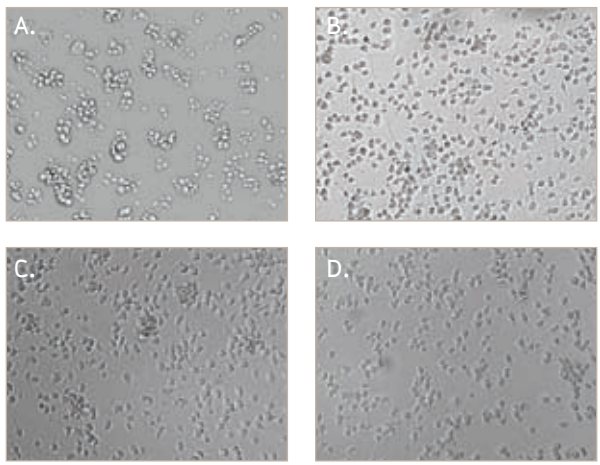

Figure 4. Brightfield image of rat NSC on different ECM substrates. Cell morphology of rat NSC when attached to (A) collagen-1, (B) fibronectin, (C) laminin, and (D) vitronectin at 20 µg/mL concentration.

Next, we examined the interactions between integrins and ECM proteins by combining functional blocking antibodies to integrins with the ECM Cell Culture Optimization Array. The major integrin receptors to collagen-1 are α1β1 and α2β1 isoforms[3], and the major integrin receptors to vitronectin are αVβ3 and αVβ5 isoforms[4]. Blocking of the individual integrin pathways was achieved by incubating cell suspension with an azide-free antibody in serum-free medium.

It was found that the anti-integrin αvβ3 antibody significantly reduced the adhesion of A-375 cells to pre-coated vitronectin, while the same concentration of anti-integrin αvβ5 antibody did not inhibit cell adhesion (Figure 6). Similarly, the antibody against collagen-1 receptor α2β1 compromised the adhesion of A549 cells to collagen-1, while the antibody against α1 integrin only slightly affected adhesion (Figure 7). When both antibodies were used together, they did not create a synergistic effect on adhesion in either cell line. This data suggests that in A-375 cells, the αvβ5 integrin is the major receptor to vitronectin, while in A549 cells, the α2β1 integrin is the major receptor for collagen-1.

Our data not only demonstrates that optimal ECM-dependent and integrin-specific cell adhesion can be achieved using the 96-well ECM Cell Culture Optimization Arrays, but also that this assay provides an important aid in the development of a physiological cell environment by providing information that reflects the cell response *in vivo*. Further applications, such as directing cell proliferation and/or differentiation may be tested using the 48-well format, which is also available through Millipore Corporation.

References

1. *Nature*. 2002 May 2;417(6884):39-44.
2. *BMC Neurosci*. 2008 Jul 23;9:71.
3. *J Biol Chem*. 1995 Jun 2;270(22):13548-52
4. *Exp Dermatol*. 1996 Dec;5(6):308-15

Related Products

Description	Catalogue No.
ECM Cell Culture Optimization Array (colorimetric, 96 wells)	ECM541
ECM Cell Culture Optimization Array (fluorometric, 96 wells)	ECM546
ECM Cell Culture Optimization Array (colorimetric, 48 wells)	ECM542
ReNcell VM Immortalized Cell Kit	SCC010
Adult Rat Hippocampal Neural Stem Cell Kit	SCR021
Anti-Integrin α1, clone FB12, azide free	MAB1973Z
Anti-Integrin α2β1, clone BHA2.1, azide free	MAB1998Z
Anti-Integrin αVβ3, clone LM609, azide Free	MAB1976Z
Anti-Integrin αVβ5, clone P1F6, azide free	MAB1961Z

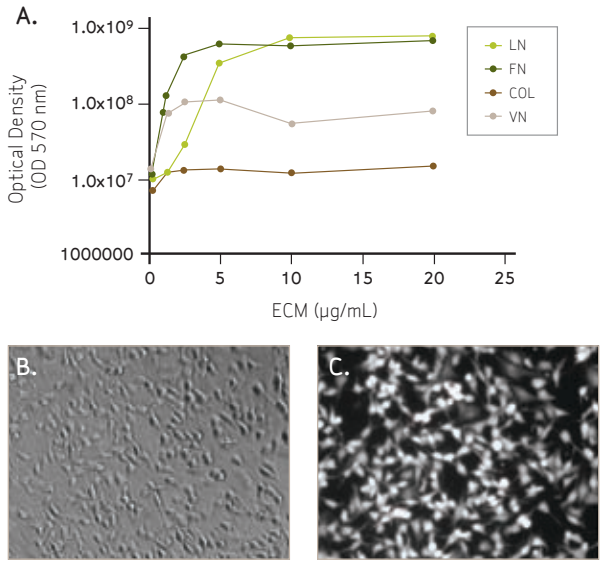

Figure 5. ReNcell VM adhesion profiling using calcein-AM staining. ReNcell VM cells were plated on the ECM cell Culture Optimization Array and stained with calcein-AM for detection. (A) The graphic presentation of triplicate data (B) brightfield image of ReNcell VM on laminin substrate at 20 µg/mL and corresponding fluorescent image at (C).

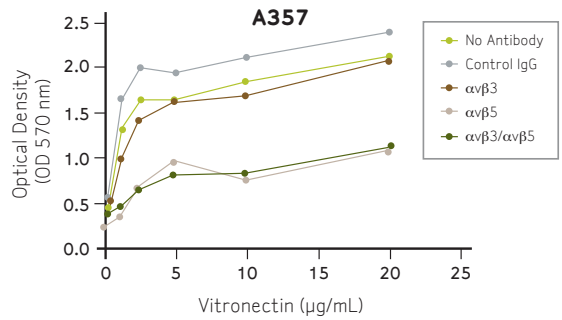

Figure 6. Effect on melanoma cell line A-375 adhesion to vitronectin with blocking antibody against integrin αVβ3 And αVβ5. 20 µg/mL antibody was used to block the target integrin in 10^6 cell/mL suspension at 37 °C for 1 hour prior applying to the ECM Cell Culture Optimization Array. The αVβ5 integrin appears to be the major integrin to interact with vitronectin in A-375 cells.

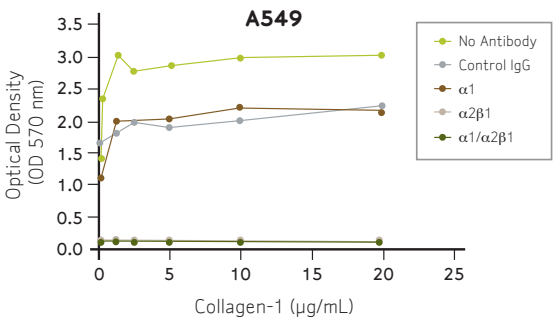

Figure 7. Effect on lung carcinoma cell line A549 adhesion to collagen-1 with blocking antibody against integrin α1 and α2β1. 20 µg/mL antibody was used to block the target integrin in 10^6 cell/mL suspension at 37 °C for 1 hour prior applying to the ECM Cell Culture Optimization Array. The α2β1 integrin appears to be the major integrin to interact with collagen-1 in A549 cells.

Enrichment of OCT4 positive pluripotent hES cells using cell surface markers

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Human embryonic stem cells (hESCs) are traditionally characterised using a panel of antibodies to cell surface markers such as SSEA-3, Tra-1-60, and Tra-1-81. However, none of these markers are entirely specific to hESCs, as they have also been shown to detect differentiated somatic cell types.[1] Another traditional marker, OCT4, is considered to be the best characterised marker for hESC cells, and is absolutely required for the development of pluripotent cell types.[1,2] However, OCT4 is a transcription factor that is found in the nucleus, and not on the cell surface of cells. Therefore, in order to detect its presence using traditional characterization methods, cells must first be fixed, which ultimately leads to cell death.

To help solve this issue, we have carried out a series of experiments using combinations of two known hESC markers: TG30 (Catalogue No. MAB4427, see Fig. 1A), which detects human CD9;[3] and TG343 or GCTM-343 (Catalogue No. MAB4346, see Fig. 1B), which detects a proteoglycan on the surface of hESCs.[4] Both of these antibodies, TG30 and TG343, have been recently validated in the International Stem Cell Initiative as good markers for hESC.[5] By combining these two cell surface markers, we were able to successfully develop a simple flow cytometric assay protocol that allows for the enrichment of live OCT4 positive cells without fixation or the construction of a fluorescent reporter cell line utilising the OCT4 promoter (protocol listed at right).

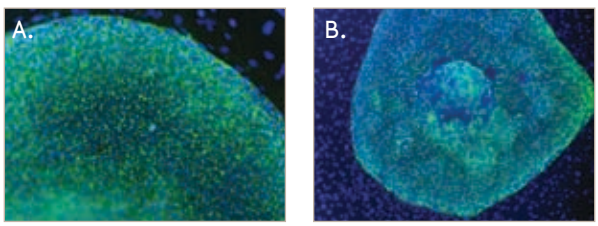

Figure 1. MEL-1 human ES cells labeled with the TG30 antibody, Cat. No. MAB4427, overlayed with DAPI (left) and (B) MEL-2 human ES cells labeled with the TG343 antibody, Cat. No. MAB4346, overlayed with DAPI (right). Only pluripotent human ES cells are labeled by the TG30 and TG343 antibodies; note that the antibodies do not recognize the scattered cells making up the feeder layer surrounding the human ES cell colony. Labeling was done via indirect fluorescence. (Images courtesy of the Australian Stem Cell Centre).

PROTOCOL

Triple staining of human embryonic stem (hES) cells for FACS (for 1x10^6 cells)

Step 1: Double-staining of two cell surface markers

(1) Harvest hES cells (MEL-1 hES cell line, SCC020; or MEL-2, hES cell line, SCC021).
(2) Wash 2x in medium (DMEM containing 20% FCS) and pellet cells.
(3) Incubate cells with 500 µL of TG30 (MAB4427) antibody and 6 µg/mL of TG343 (MAB4346) antibody for 30 minutes on ice.
(4) Wash 2x in ice-cold medium; pellet cells.
(5) Incubate with FITC-goat anti-mouse IgG_{2a} (1:500) and APC-goat anti-mouse IgM (1:1000) in medium for 30 minutes on ice.
(6) Wash 2x in ice-cold medium; pellet cells.

Step 2: Fixation and permeabilization of hES cells, and the staining of the third (intracellular) marker

(1) Resuspend cells in 1 mL of 2% PFA (in PBS) for 30 minutes at room temperature, mixing occasionally.
(2) Wash 2x in ice-cold medium; pellet cells.
(3) Resuspend cells in 1 mL of 0.1% Triton-X for 5 minutes at room temperature.
(4) Wash 2x in ice-cold medium; pellet cells.
(5) Resuspend cells in 1 mL of 10% goat serum for 30 minutes at room temperature.
(6) Incubate with OCT4 antibody (MAB4401) in 10% normal goat serum (4 µg/mL) for 30 minutes on ice.
(7) Wash 2x in ice-cold medium; pellet cells.
(8) Stain with PE-goat anti-mouse IgG_1 in 10% goat serum (1:2000) for 30 minutes on ice.
(9) Wash 2x in ice-cold medium; pellet cells.
(10) Resuspend in 10% goat serum for FACS. (NOTE: Try to avoid using reagents that contain phenol red or PI staining, which might increase the background for fixed cells).

As described below, human embryonic stem cells were first labeled with the two cell surface markers, TG30 and TG343, and then fixed prior to OCT4 staining. A single-color flow cytometric assay was then carried out using an OCT4 antibody (Millipore, MAB4401), and showed that ~70% of these cells were positive for OCT4 (data not shown). In contrast, >90% of cells that were brightly positive for both TG30 and TG343 were also positive for OCT4 (Figure 1). Therefore, we have found that this procedure provides a robust and rapid methodology to enrich for live, OCT4-positive cells from a heterogenous mixture of undifferentiated and differentiated cell types.

References

1. Laslett, A. L., *et al.*, 2003. Characterization and culture of human embryonic stem cells. *Trends Cardiovasc Med.* **13**, 295-301.
2. Nichols, J., *et al.*, 1998. Formation of pluripotent stem cells in the mammalian embryo depends on the POU transcription factor Oct4. *Cell.* **95**, 379-91.
3. Laslett, A. L., *et al.*, 2007. Transcriptional analysis of early lineage commitment in human embryonic stem cells. *BMC Dev Biol.* **7**, 12.
4. Cooper, S., *et al.*, 2002. Biochemical properties of a keratan sulphate/chondroitin sulphate proteoglycan expressed in primate pluripotent stem cells. *J Anat.* **200**, 259-65.
5. Adewumi, O., *et al,* 2007. Characterization of human embryonic stem cell lines by the International Stem Cell Initiative. *Nat Biotechnol.* **25**, 803-16.

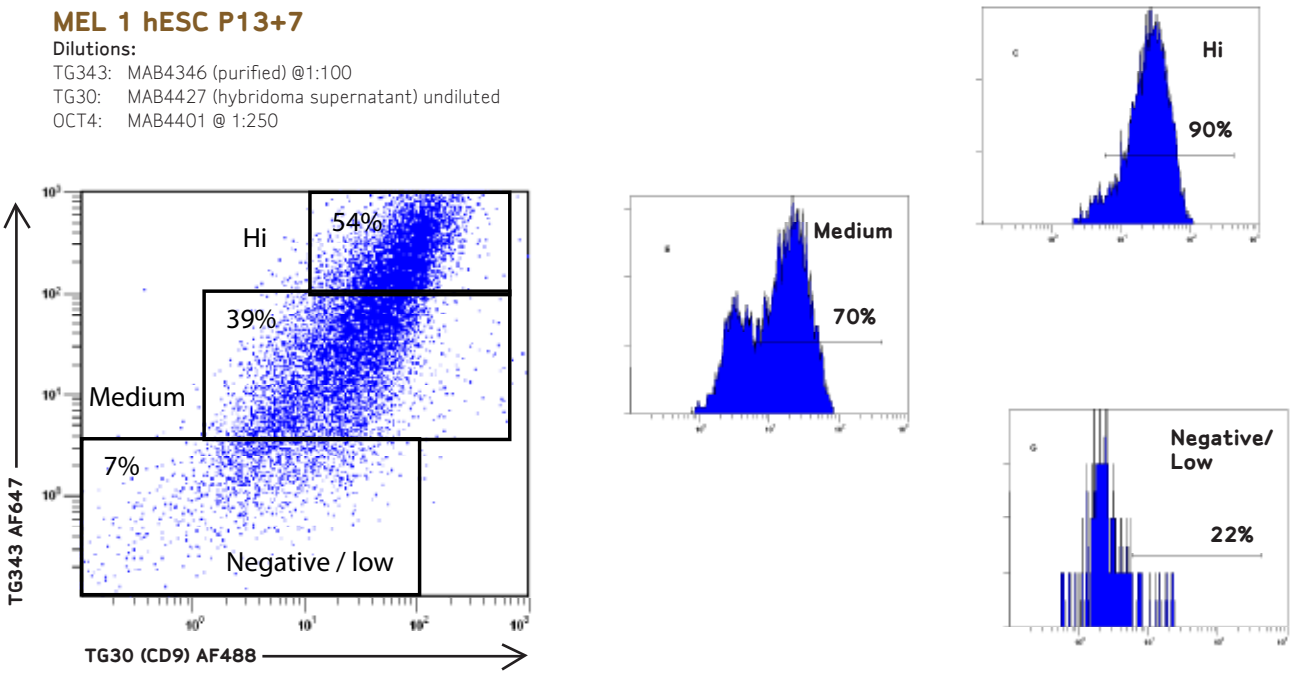

Figure 1. Combined flow cytometric analysis of hESC for TG343 (MAB4346), TG30 (MAB4427) and OCT4 (MAB4401). Gates are set relative to isotype controls. Right hand panel: Percentages of cells staining for OCT4 from regions negative/low, medium and high from left hand panel. Cells showing high immunoreactivity to both TG343 and TG30 antibodies are 90% positive for OCT4.

Related Products

Description	Qty	Catalogue No.
Anti-TG30, clone TG30	100 µg	MAB4427
Anti-TG343, clone TG343	100 µg	MAB4346
Anti-OCT4, clone 10H11.2	100 µg	MAB4401
MEL-1 Human Embryonic Stem Cells	1 kit	SCC020
MEL-2 Human Embryonic Stem Cells	1 kit	SCC021
Anti-HESCA-1, clone 051007-4A5	100 µg	MAB4407
Anti-HESCA-2, clone 060818-7A6	100 µg	MAB4406
Anti-Stage Specific Embryonic Antigen-3	100 µg	MAB4303
Anti-Stage Specific Embryonic Antigen-4	100 µg	MAB4304
ES Cell Characterization Kit	100 assays	SCR001

NEW PRODUCTS

MilliTrace GFP Reporter Neural Stem Cell Lines

Millipore provides ready-to-use primary neural stem cells that are constitutively labeled with humanized mulleri GFP (hmGFP). FACS analyses of the stable transfectants indicate that over 95% of the cells express GFP at high levels even after 10 passages. In addition, these cells display the immunocytochemical staining properties of neural stem cells. Under conditions favoring differentiation, they give rise to high numbers of neurons and astrocytes while maintaining GFP expression, thus demonstrating their multipotentiality.

Description	Catalogue No.
MilliTrace Constitutive GFP Reporter Adult Rat Hippocampal NSC Kit	SCR080
MilliTrace Constitutive GFP Reporter Mouse Cortical NSC Kit	SCR081
MilliTrace Rat Neural Stem Cell Expansion Medium	SCM040
MilliTrace Mouse Neural Stem Cell Expansion Medium	SCM041
MilliTrace Rodent Neural Stem Cell Basal Medium	SCM040

ESGRO Complete™ C57/BL6 Mouse Embryonic Stem Cell Line

The ESGRO Complete C57/BL6 mouse embryonic stem (ES) cell line has been pre-adapted to growth in serum-free and feeder-free cell culture conditions, reducing the time associated with switching cells over to the ESGRO Complete ES cell medium. Confirmed to be germline competent, these mouse ES cells can be used for the creation of animal models or for basic research/differentiation studies.

Description	Catalogue No.
ESGRO Complete C57/BL6 Mouse Embryonic Stem Cell Line, 2 vials, 2.5 x 10^6 ea	SF-CMTI-2

Recombinant Human IGF-I and Mouse Stem Cell Factor

Millipore is pleased to release two new growth factors: human insulin-like growth factor-I (IGF-I), a polypeptide growth factor which stimulates the proliferation of a wide range of cell types including muscle, bone, and cartilage tissue; and mouse Stem Cell Factor (SCF), a hematopoietic growth factor that exerts its activity at the early stages of hematopoiesis. Our recombinant growth factors are thoroughly tested for bioactivity, purity, and endotoxin levels. Bulk sizes are available for large scale studies.

Description	Catalogue No.
Recombinant Human Insulin-Like Growth Factor I (IGF-I),100 µg	GF138
Recombinant Mouse Stem Cell Factor (SCF),10 µg	GF141

Human Embryoid Body Formation Medium

This medium is designed to support the spontaneous or directed differentiation of human embryonic stem cells. The medium can be used to form embryoid bodies in suspension culture on low adhesion plates. Embryoid bodies formed using SCM026 medium have been shown to facilitate the differentiation of human ES cells into neural, endodermal, and cardiac cell lineages.

Description	Catalogue No.
Human Embryoid Body Formation Medium	SCM026

Collagenase Type I: Validated Passaging Agent for hES cells

This validated enzyme is uniquely optimized for the successful enzymatic passaging of hES cells cultured with Millipore's HEScGRO™ medium. It is an excellent alternative to collagenase type IV, which is not compatible with HEScGRO medium, and provides hES researchers with another robust option for enzymatic passaging and xeno-free culture of hES cells.

Description	Catalogue No.
Collagenase Type I, 250 mg	SCR103

Epidermal Keratinocyte 3D Medium

This revolutionary medium was created to address the specific needs of 3D cell cultures. When used in conjunction with CELLnTEC human primary keratinocytes and Millipore's Millicell inserts, you can develop accurate human epidermal models that contain multiple epidermal layers (stratum corneum, granulosum, spinosum, and basale), and also maintain these *in vitro* cultures beyond 28 days. The new 3D media is a fully defined, serum-free, BPE-free formulation, and is the only commercially available medium specifically formulated and optimized for 3D keratinocyte culture. See article in this issue on page 6 for more details.

Description	Catalogue No.
Epidermal Keratinocyte 3D Medium, Defined, 100 mL	CnT-02-3D1
Epidermal Keratinocyte 3D Medium, Defined, 500 mL	CnT-02-3D5

ShSCP-5 Antibody, clone 8H9.3 - Pluripotent hESC Marker

Developed in collaboration with Axordia Ltd./University of Sheffield, anti-ShSCP-5 is a novel stem cell antibody that recognizes a potentially unique 50 kDa cell surface protein, which has been found to be specifically expressed on undifferentiated human embryonic stem cells and embryonal carcinomas. This monoclonal antibody has been validated in immunocytochemistry and Western blotting applications.

Description	Catalogue No.
ShSCP-5 Antibody, clone 8H9.3	MAB4408

TG30 Antibody, clone TG30 - Pluripotent hESC Marker

The monoclonal TG30 antibody recognizes an epitope of a cell surface protein that has been further identified as CD9. When this antibody is used in conjunction with the TG343 antibody in flow cytometry, it works as a robust and rapid tool for the purification and selection of live OCT4-positive human embryonic stem cells from mixed cell populations. This antibody has been validated in immunocytochemistry, flow cytometry, and immunofluorescence applications.

Description	Catalogue No.
TG30 Antibody, clone TG30	MAB4427

TG343 Antibody, clone TG343 - Pluripotent hESC Marker

The monoclonal TG343 antibody recognizes a distinct epitope on the protein core of a pericellular matrix protein that is also recognized by GCTM-2. When used in conjunction with the TG30 antibody, it works as a robust and rapid tool for the purification and selection of live OCT4-positive human embryonic stem cells from mixed cell populations. This antibody has been validated in immunocytochemistry, flow cytometry, immunofluorescence, and Western blotting applications.

Description	Catalogue No.
TG343 Antibody, clone TG343	MAB4346

Anti-ID2, clone 10C5.2

ID2 plays a key role in the regulation of lineage commitment, cell fate decisions, and in the timing of differentiation during neurogenesis, lymphopoiesis, and angiogenesis. The ID protein family, which is induced by the bone morphogenic protein-SMAD pathway, has also been shown to collaborate with LIF to ensure that embryonic stem cells opt for self-renewal. This monoclonal antibody has been validated in immunohistochemistry and Luminex® applications.

Description	Catalogue No.
Anti-ID2, clone 10C5.2	MAB4358

Human ESC Germ Layer PCR Kit

Characterizing the potential of hESCs to differentiate into the three germ layers is a critical step to assess their pluripotent capability. Millipore's Human ESC Germ Layer PCR Kit provides an easy to use molecular tool to analyze hESCs in their pluripotent or differentiated stages. It contains primer sets for all pluripotent and lineage markers, and control cDNA for both human ES cells and embryoid bodies.

Description	Catalogue No.
Human ESC Germ Layer PCR Kit, 100 reactions	SCR063

33 mm Sterile GP and HP Express PLUS (PES) Millex® Syringe Filters, the fastest filters on the market!

The new 33 mm Millex syringe filters with Millipore Express® PLUS (PES) membrane are available in 0.22 µm (GP) and 0.45 µm (HP) pore sizes offering, you more membrane surface area. The color-coded filters with an over-molded design allow a high operating pressure of 150 psig (10 bar) and deliver faster filtration flow rates than the standard 25 mm devices.

Description	Catalogue No.
Millex-GP 33 mm Filter, 0.22 µm Millipore Express PLUS, gamma-sterilized	SLGP033RS
Millex-GP 33 mm Filter, 0.22 µm Millipore Express PLUS, gamma-sterilized	SLGP033RB
Millex-HP 33 mm Filter, 0.45 µm Millipore Express PLUS, gamma-sterilized	SLHP033RS
Millex-HP 33 mm Filter, 0.45 µm Millipore Express PLUS, gamma-sterilized	SLHP033RB
Medical Millex-HP 33 mm Filter, 0.45 µm Express PLUS, gamma-sterilized	SLHPM33RS
Medical Millex-GP 33 mm Filter, 0.22 µm Express PLUS, gamma-sterilized	SLGPM33RS

MultiScreen®$_{HTS}$+ Hi Flow Filter Plates

For your most critical applications, choose the filter plate that will improve sensitivity and increase flow: Millipore's new MultiScreen$_{HTS}$+ Hi Flow filter plates. Designed for radioactive bio-assays, the new plate uses a mesh-supported membrane to create uniformly flowing wells and improve washing efficiency. The new MultiScreen$_{HTS}$+ plate design also lessens non-specific binding and reduces variability in both background and signal intensities. Testing showed a three-fold decrease in well-to-well variability and improved signal-to-noise ratio over traditional filter plates. You'll also achieve higher throughput, greater assay sensitivity, and flexibility in detection.

Description	Catalogue No.
MultiScreen$_{HTS}$+ Hi Flow FB Plate, opaque, non-sterile, 96-well	MSFBNXB50
MultiScreen$_{HTS}$+ Hi Flow FC Plate, opaque, non-sterile, 96-well	MSFCNXB50
MultiScreen$_{HTS}$+ Hi Flow PH Plate, opaque, non-sterile, 96-well	MSPHNXB50

Millicell Cell Culture Receiver Plates

Millipore's Millicell family of products offers numerous technical advantages over plastic plates, especially for epithelial and endothelial cell cultures and assays. Millicell cell culture plates are high quality injection molded polystyrene plates that are tissue culture treated and provided sterile for use in direct and co-culture applications. The plates are engineered to fit with Millipore's hanging and standing cell culture inserts.

Description	Catalogue No.
Millicell Cell Culture Receiver Plate, tissue culture treated, sterile, 50/pk, 6 well	PIMWS0650
Millicell Cell Culture Receiver Plate, tissue culture treated, sterile, 50/pk, 12 well	PIMWS1250
Millicell Cell Culture Receiver Plate, tissue culture treated, sterile, 50/pk, 24 well	PIMWS2450

NEW PRODUCTS

FlowCellect™ Kits

Millipore's new FlowCellect kits for stem cell characterization are designed to provide rapid, sensitive assessments of embryonic and neural stem cell phenotypes at various stages of differentiation. These robust flow cytometry kits will make your research faster, easier and more accurate than ever before.

Description	Catalogue No.
Human Embryonic Stem Cell (OCT-4) Nuclear Marker Characterization Kit	FCHEC25102
Human Embryonic Stem Cell (HESCA-1) Surface Marker Characterization Kit	FCHEC25104
Human Embryonic Stem Cell (TRA-1-60) Surface Marker Characterization Kit	FCHEC25106
Mouse Embryonic Stem Cell (OCT-4) Nuclear Marker Characterization Kit	FCMEC25110
Rodent Neural Stem Cell Characterization Kit (Neural Differentiation)	FCRNC25112
Rodent Neural Stem Cell Characterization Kit (Astrocyte Differentiation)	FCRNC25114

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