HOW TO TRANSFER

from animal-derived matrices to GrowDex – 3D cell culture matrix

PerkinElmer
For the Better

An Authorized Distributor

Contents

1. Introduction

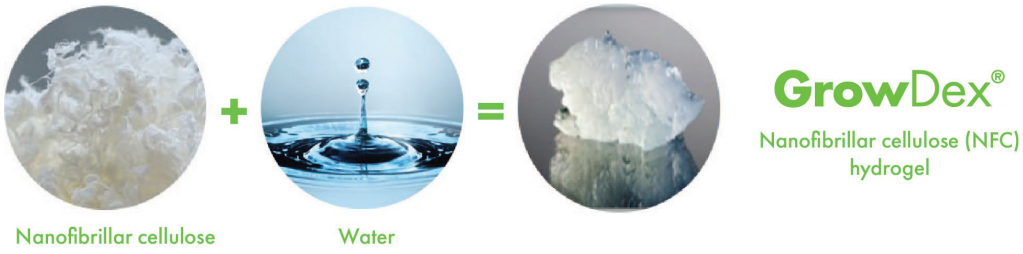
Animal-derived matrices are often poorly defined, contain contaminating molecules, and suffer from lot-to-lot variability. This unnecessary variability ultimately effects the experiment reproducibility, which is an essential element for reliable research studies.

Natural, reliable and ready-to-use, GrowDex® is the next generation solution for reproducible and scalable animal-free 3D cell culture applications providing consistent results with convenience. GrowDex is produced from wood-derived nanofibrillar cellulose (NFC) which guarantees its pure and clean structure as well reproducible lots.

Replacing animal-derived products with GrowDex is simple and easy. Read more below about why and how to transfer to GrowDex from animal-derived matrices.

2. What is GrowDex made of?

GrowDex is an animal-free, ready-to-use hydrogel that mimics the extracellular matrix (ECM) and supports cell growth and differentiation with consistent results. GrowDex is made from wood-derived nanofibrillar cellulose (NFC) and water, which guarantees its pure and clean structure as well reproducible lots. It does not contain any animal-derived components or have any unknown factors, just nanocellulose fibers and water.



3. What are animal-derived matrices made of?

The most commonly used animal-derived matrices for 3D cell culture are extracts of Engelbreth-Holm-Swarm mouse tumours and animal-derived collagen (e.g. rat tail collagen). These matrices are composed of proteins such as collagen, laminin and entactinentactin and unknown concentrations of growth factors and other proteins. Hughes et. al., (2010) [1] performed an in-depth proteomic analysis of Matrigel. They reported to have identified 1851 unique proteins and concluded that the composition of Matrigel is a complex and intricate mixture of structural proteins, growth factors and their binding proteins, as well as other proteins of roles which are still not clear in cell culture. Additionally, it is well known that there are lot-to-lot and supplier-source variability of Matrigel, which has a strong impact on cell behaviour and fate [2-4]. Hence, the exact composition is unknown, and the composition varies between lots. The above listed factors have an impact on cell culture performance and might cause unexpected cell behaviour, for example when human cells are cultured in animal-based matrices. The next chapter will show an example of HepG2 cells cultured in an animal-free matrix GrowDex compared to an animal-derived matrix Matrigel.

4. A working example: Cell performance comparing the 3D cell culture of HepG2 cells in GrowDex and Matrigel

This section will detail an example where HepG2 cells were cultured in GrowDex and Matrigel [31]. The specific experiment setup details, results, and suggested protocols of how to transfer current Matrigel workflows are also detailed below.

4.1. Experimental Setup

HepG2 cell viability was compared between three different lots of GrowDex and growth factor reduced Matrigel. The protein concentration in Matrigel lots varied between 7.5 – 8.9 mg/ml. Please see culturing details in Table 1 below. For each lot of Matrigel and GrowDex, 5 replicates of cell viability were measured at each time point and 3 independent experiments were performed.

Table 1: HepG2 cell culture set-up

	GrowDex	Matrigel
Cells/well	8 000	8 000
Culture type	Embedded	Embedded
Well plate	96 well ULA plate (#3474, Corning)	96 well ULA plate (#3474, Corning)
Matrix dilution	0.3% GrowDex diluted with DMEM (41966-029, Gibco) supplemented with 10% FBS and 1x pen/strep	50% Matrigel diluted with DMEM (41966-029, Gibco) supplemented with 10% FBS and 1x pen/strep
Cells and matrix per well	100 µl	100 µl
Media on top (µl)	100 µl (added directly)	100 µl (added after 15 min polarization time)
Readout	Cell Titer Glo (CTG) 2.0 (cat. G9243), luminescence measurement	CTG 2.0 (cat. G9243), luminescence measurement

4.2. Results

The cell viability results (Figure 1, A-B) showed that GrowDex provides a more consistent and reproducible extracellular environment for the HepG2 cells, with less standard deviation between replicate wells and repeated experiments when compared to three different Matrigel lots. Notably, following 96h in culture, the cell viability according to CTG measurement was significantly higher ($p<0.0001$) in GrowDex in comparison to Matrigel (Figure 1, B).

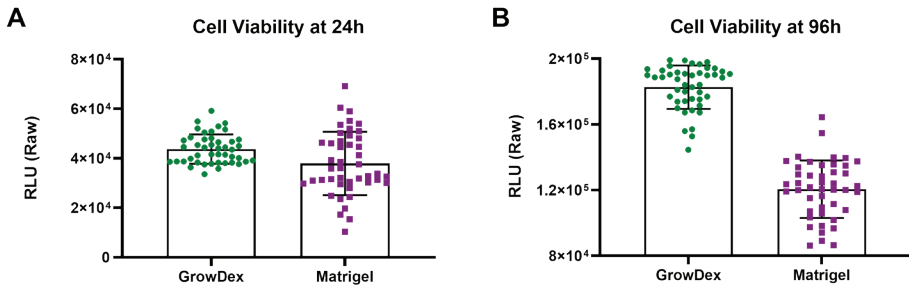


Figure 1: Cell viability of HepG2 cells cultured in GrowDex and Matrigel. The data shows the viability of HepG2 cells measured by the CTG assay after 24h (A) and 96h (B) of culture in GrowDex (green dots) or Matrigel (purple squares). Data is shown as a scatter plot with the Mean ± SD measured from cells cultured in three different lots of hydrogel, with 5 repeat wells per lot, repeated 3 times.

When calculating the viability ratio, i.e. the change in cell viability specifically between 24h through 96h of cell culture, there was little variation between test sample replicates and lots (Figure 2, A) when cells were cultured in GrowDex. On the other hand, there was a lot of variation in the viability ratio of test sample replicates and lots for cells cultured in Matrigel, as seen in Figure 2, B.

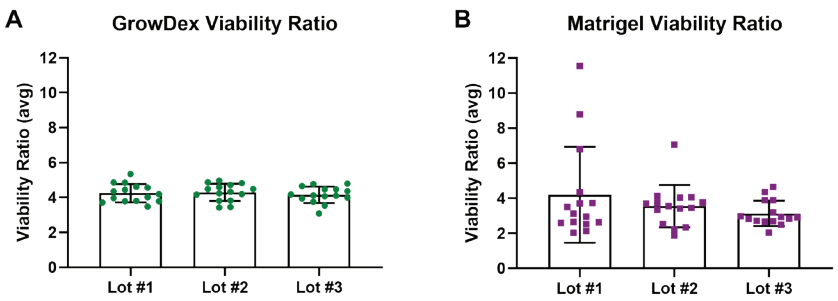


Figure 2: Cell viability ratio in different GrowDex and Matrigel lots. The cell viability ratio of HepG2 cells was calculated from 96h/24h viabilities in GrowDex (A) and Matrigel (B). Data is shown as a scatter plot with the Mean ± SD measured from cells cultured in three different lots of hydrogel, with 5 repeat wells per lot, repeated 3 times.

Microscopy images (Figure 3) showed that the HepG2 cells formed into spheroids when embedded in the GrowDex hydrogel. These spheroids had a more spherical and homogenous morphology. On the other hand, spheroids formed in Matrigel showed random shaped morphology, uncontrolled growth, and spheroid merging. This may be a result of the presence of unknown concentrations of ECM proteins, growth factors and other biological signalling molecules in the material that has also been observed by Niklander et al. [5].

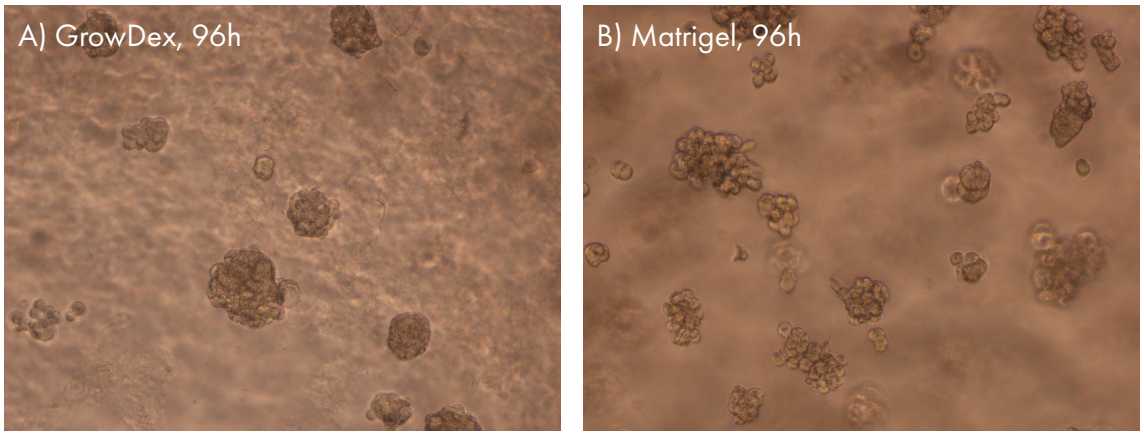


Figure 3: Microscopy images of HepG2 spheroids in GrowDex and Matrigel. Following 96h of culture in either GrowDex (A) or Matrigel (B) HepG2 cells were seen to form spheroids throughout the hydrogel. Nikon Eclipse, 10x.

5. Switching from animal-derived matrices to GrowDex

The table below (Table 2) shows a comparison of features and compatible regents between GrowDex and animal-derived matrices. This table is followed by chapters explaining each aspect which you need to be aware of when transitioning from an animal-derived matrix to animal-free GrowDex. These chapters cover handling techniques, working temperature, dilution with media, stiffness tunability, storage conditions, autofluorescence, pipette tips and cell recovery.

Table 2: Key differences between GrowDex and animal-derived matrices

	GrowDex	Animal-derived matrix
Handling temperature	Room temperature	<4 °C
Polarization temperature	Not required	22 – 37 °C
Compatible dilution solution	Any media	Dilute with PBS or media
Stiffness tunability	GrowDex stiffness is within the low kPa range (ca. 0-2000 Pa). This can be fine-tuned by diluting with culture media.	Unknown
Storage	Unopened: room temperature opened: 4 – 8 °C	- 20 °C
Autofluorescence	Non-auto fluorescent	Proteins cause background fluorescence
Compatible pipette tips	Low-retention and low-adhesion pipette tips	Pre-cooled (< 4 °C) pipette tips
Cell recovery reagent	GrowDase (cellulase enzyme)	Proteolytic enzymes, dispase or Corning Cell Recovery Solution
Purity	RNA/DNA free	Traces of animal RNA/DNA

5.1. Handling and working temperature

GrowDex is temperature stable hydrogel allowing room temperature handling without any changes in the mechanical properties of the gel, even if the temperature would change during the cell culture assay workflow. Since GrowDex is delivered as a ready-to-use hydrogel, without the need for cross-linking or gelation, it is easy to work with and fast to incorporate into current workflows.

The shear-thinning material property of GrowDex means that the product is a semi-solid and a highly viscous hydrogel at rest, but when a shear force is applied, it becomes temporarily fluid. Immediately after the shear-force is removed, GrowDex returns to a semi-solid hydrogel again. Shear force is generated, for example during pipetting or when injecting GrowDex from a syringe. The shear-thinning property of GrowDex makes it possible to dilute the product with any liquid and tune the stiffness of the gel as desired. Once GrowDex has been diluted, it will again remain as a semi-solid hydrogel, however, in a looser form than as the stock concentration.

In comparison, the stiffness of **animal-derived matrices** vary depending on the temperature. Animal-derived matrices are solid gel between temperatures 22°C to 37°C. At temperatures below 4°C they liquify or become solutions. Adjusting the stiffness of them might become challenging as it is difficult to accurately control the solidification process. This poses several practical difficulties, such as dispensing, reproducibility and matching the stiffness to the cells' tissue type. The unexpected solidification of the animal-derived matrix due to temperature changes causes clogging of pipette tips and will influence the reproducibility of assays. Also, finetuning the cell microenvironment and the stiffness to match the tissue type might become a challenge as slight changes in temperature might cause changes in the stiffness that the cells experience inside it.

5.2. Tuning the stiffness

GrowDex's tunability provides an opportunity to customize the cellular environment to match the tissue or cell type under investigation. The shear-thinning property of GrowDex makes it possible to dilute the stock gel (GrowDex stock concentration is 1.5%) into different working concentrations. This allows the user to fine tune the microenvironment to suit their cells and achieve the optimal stiffness found in the tissue in vivo. The graph below (Figure 4) shows the stiffness (storage modulus, Pa) measured for GrowDex from 0.3% to 1 % stiffness. The analysis was performed with rotational rheometer. Please note that other analysis methods may give different result.

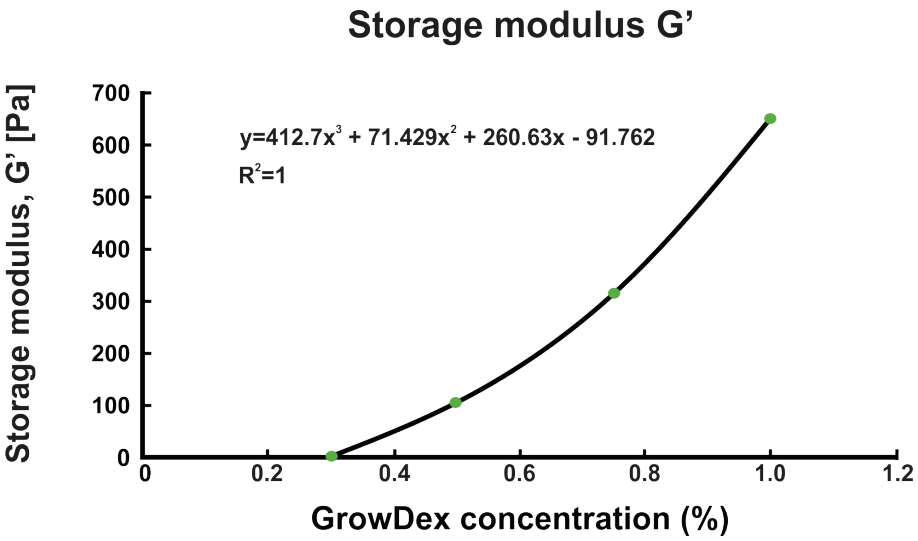


Figure 4: Storage modulus (G') measurements relative to GrowDex concentration. The analysis was performed with rotational rheometer measuring the stiffness of GrowDex at 0.3%, 0.5% 0.75% and 1.0%. It should be noted that other analysis methods may give different results. It can be seen that GrowDex stiffness is within the low kPa range. The stiffness of 1kPa can be extrapolated with the equation shown in the graph.

Animal-derived matrices, however, are not conducive to manipulation, making it more difficult to fine-tune and achieve specific biological outcomes [2]. Tuning the stiffness of animal-derived matrices is based on the temperature change, which causes them to turn from liquid to solid matrices. As a result, animal-derived matrices cannot easily match the tissue-specific niches required to control and direct specific programmes of stem cell differentiation [3]. Similarly, collagen-based hydrogels have presented a non-optimal stiffness and low rigidity which may affect the cell growth and morphology.

5.3. Storage & Thawing

GrowDex can be stored in room temperature, it is ready-to-use and the properties of GrowDex will not change, even if the temperature would fluctuate during your assay. We recommend storing GrowDex at 4-22°C (39–72°F) and protected from light for optimum performance. Once it has been opened, we recommend that the product is stored undiluted at 4-8°C (39-46°F) for a maximum of 3 months. For convenient working schedule, GrowDex can be diluted with cell culture medium in beforehand. The diluted hydrogel should be stored at 4-8°C (39-46°F) for a maximum of 7 days and it can be used immediately for starting the cell cultures. Freezing will result in destabilization of the product rendering it unusable. Thus, the product should not be stored below 0°C (32°F). Since GrowDex is stored and used in room temperature, there is no need for thawing or aliquoting.

Animal-derived matrices require cold temperature storage that in turn leads to long preparation steps, once new assays are started with the product. The storage temperature for animal-derived matrices is -20°C (4°F) or -70°C (-94°F). The need for low temperatures demands cautious storing and adds additional steps to the use and preparation of animal-derived matrices. If the whole animal-derived matrix vial is required for the assay, it should be thawed overnight on ice in a 2-6°C (36-43°F) at the back of a refrigerator, or in a cold room thus requiring special caution, when taking the product into use. As animal-derived matrices also do not tolerate repeated freeze-thaw cycles, they must be aliquoted into separate storage containers, so that the product can be used in smaller quantities as needed. After aliquoting the animal-derived matrix is done, the aliquoted vials can be used after one-hour thawing. To conclude, preparing new assays with animal-derived matrices require the user to take in consideration the preparation steps needed to start new assays and thus affects the flexibility to change or run assays in the lab.

5.4. Lot-to-lot consistency: Protein concentration & growth factors

GrowDex hydrogels have reproducible and quality-controlled lots that makes it possible to repeat assays with consistent cell behaviour and differentiation. GrowDex does not have lot-to-lot variation and all the batches are tested with cell culture assays and mechanical tests to assure the highest quality and consistency. GrowDex is produced from wood-derived nanofibrillar cellulose (NFC), therefore, it does not include any traces of animal DNA or RNA. UPM Biomedicals' NFC is manufactured according to ISO 13485 Medical Devices Quality Management system and the product biological safety has been evaluated according to 'ISO 10993 - Biological evaluation of medical devices': genotoxicity and cytotoxicity.

On the other hand, every lot of **animal-derived matrices** have a lot-specific protein concentration or recommended dilution factor, and therefore there is always lot-to-lot variation. This will result in the need to pre-screen and pre-select batches that are suitable for specific cell types and acquire large enough production lots to make it possible to run assays with consistent results within one batch. This does not rule out the variation that exists within each lot and the unknown consistency of the animal-derived matrix. It is known that besides variation in the protein concentration, there are many proteins found in animal-derived matrices which are either unknown or undefined for the user and therefore these can affect cell phenotype and differentiation potential. The main proteins that are found in animal-derived matrices are laminin, collagen and entactin. There are also many growth factors found in them including transforming growth factor beta (TGF-beta), epidermal growth factor (EGF), insulin-like growth factor (IGF-1), basic fibroblast growth factor (bFGF), and platelet-derived growth factor (PDGF) as reported by [6]. Unfortunately, it was also seen that there is an apparent increased abundance of large structural proteins in growth factor reduced animal-derived matrices in contrast to small intracellular species identified in standard animal-derived matrices [1]. Therefore, due to these variations, every animal-derived matrix lot must be pre-screened and cell culture tested, requiring valuable time and resources, as well as acquiring or batch reserving specific lots from the supplier. This is not necessary with GrowDex products.

5.5. Autofluorescence

GrowDex hydrogels contain only nanofibrillar cellulose and ultrapure water and are therefore not autofluorescent (Figure 5). Fluorescence spectroscopy –based imaging is feasible due to lack of autofluorescence. The properties of GrowDex has been studied by Bhattacharya et. al., (2012) [7].

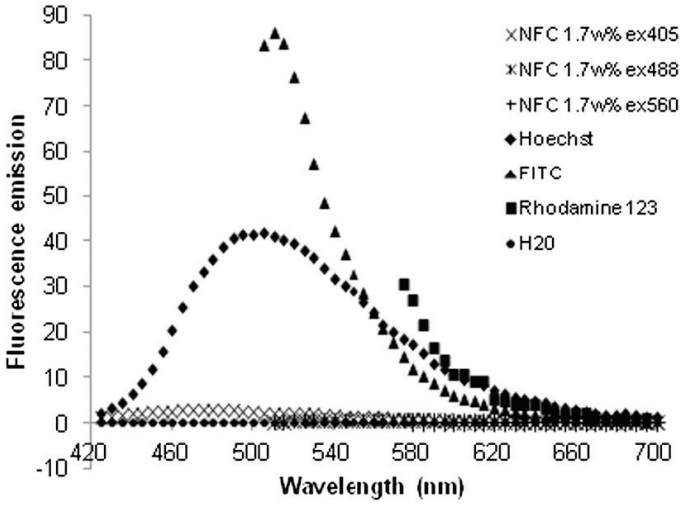


Figure 5: Optical properties of NFC, figure from [7]. Fluorescence spectra of hydrogels compared to purified water at excitation wavelengths of commonly used molecular probes (405, 488 and 560 nm).

Animal-derived matrices on the other hand, contain proteins and substances such as vitamins that may fluorescence and interfere with the experiment and readouts. For animal-derived matrices, background fluorescence needs to be determined with a control measurement that must be conducted for each experiment. As the protein concentration varies, this must be adjusted for each matrix lot acquired. Also, in the case the cells are collected from animal-derived matrices, there might be residues of the animal-derived matrix left that will interfere with the readouts and cause additional challenges in using high-content screening instruments for cell analysis.

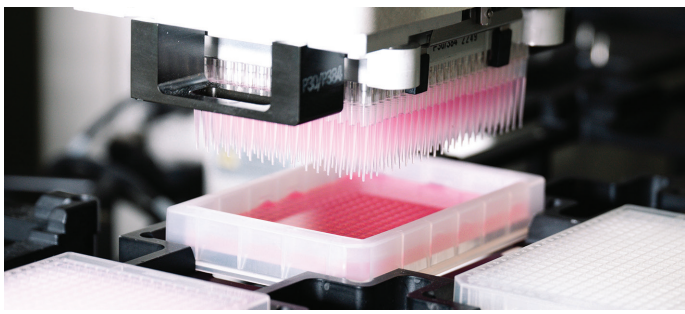
5.6. Pipetting and Pipette tips

Pipetting of **GrowDex** can be easily done in room temperature accurately and reproducibly. When pipetting GrowDex, low-retention pipette tips should be used to avoid GrowDex sticking to the tip. Low-adhesion pipette tips, e.g. TipOne RPT 1250ul CAT NO S1161-1820. Pipette tips can be cut for better flow in the beginning of mixing and then exchanged for uncut tips to increase mixing efficiency. Using a wider bore pipette tip can also help with the initial mixing step. Aspirating and dispensing GrowDex should be performed slowly to avoid air bubbles and to ensure an accurate volume. A positive-displacement pipette is useful for pipetting viscous materials like undiluted GrowDex. A multi-stepper pipette or automated dispensing system is recommended for repeat dispensing of GrowDex into the well-plates for high throughput applications as demonstrated previously [8, 9].

With **animal-derived matrices**, all the materials needed for handling the matrix, needs to be chilled before they can be used with the animal-derived matrix. The animal-derived matrix will start to crosslink and form a highly viscous gel above 10°C causing the matrix to stick to the inside and outside of warm pipette tips, well plates and vials, if they have not been properly cooled before use. This makes pipetting the matrix very challenging and will in many cases lead to excessive waste and dead volumes. In conclusion, animal-derived matrices require extra time and care, as all of the disposables used in the assays have to be pre-cooled before use and avoid the heating up of any plastics that are in touch with the matrices.

5.7. Automated liquid dispensing

As **GrowDex** is a shear-thinning and temperature stable material, it can be handled with automated dispensing systems in room temperature and it is compatible for high throughput screening applications. This has been demonstrated with a variety of different dispensers, such as Biomek NXP, Biomek FXP, Gyger Certus Flex, Eppendorf EpMotion, Labsystems Echo 525, and ThermoFisher Multidrop plate dispenser [9].



Animal-derived matrices instead require complex workflow setups where the liquid dispensing equipment must be chilled to around 4°C or using cooled plate carriers to avoid the matrix solidifying and clogging the pipette tips or tubing of the dispensers. Apart from leading to difficulties with dispensing and handling of cells, automated liquid handling and limited applications for high throughput screening, this property of the matrix clogging can lead to more variation in the setup and results, therefore making repeatable assays difficult to achieve.

5.8. Compatible media for diluting

GrowDex can be diluted with any cell culture media to provide the required nutrients and growth factors for cells in any temperature above 0 °C. With GrowDex you can choose the required culture medium and precisely control the different components added to the culture system. This makes it possible to scale up assays into HTS/HCS and make repeatable assays without having any unknown components in the culture system. Choosing the right growth factors is discussed later on in Chapter 6.

Animal-derived matrices are diluted with ice-cold culture medium. When you are diluting the animal-derived matrix into medium, the medium itself must be ice cold, or otherwise the matrix will solidify unexpectedly. If you're using the animal-derived matrix for differentiation assays you might need to use the growth factor reduced matrix to achieve higher control of growth factors and to push cell differentiation by adding them in the culture medium. Though, also the growth factor reduced version of the matrix contains unknown consistencies of different growth factors and controlling the amounts might become challenging.

5.9. Cell recovery for downstream analysis

GrowDase is a mixture of cellulase enzymes, which digests only the nanocellulose fibres of GrowDex. 3D cell structures, such as spheroids, organoids or tissue biopsies are retained with no impact on cell viability, cell surface proteins or functionality. GrowDase enzyme degrades GrowDex hydrogel into soluble sugars during a one-step efficient cell recovery process. GrowDase incubation is carried out at 37 °C. Liberated cells can be used in many downstream applications, such as gene or protein expression studies or for future cell culture experiments.

Animal-derived matrices are degraded with proteolytic enzymes (e.g. collagenase), trypsin, dispase or specialized recovery solutions. Typically, this must be performed with solutions at 4°C, or the whole culture stored at 4 °C, which can lead to changes in cell behaviour and phenotype. These reagents may also break down organoid structures and affect cell surface proteins that are found both on the cells and within the matrix.

6. Setting up the assay with GrowDex

See below nine steps to start your 3D cell culture assay with GrowDex. To count needed volumes (GrowDex, cell media, cell suspension volume).

1. Calculate the needed volumes of hydrogel, medium and cell suspension

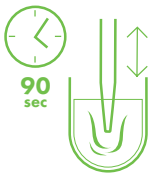
2. Pipette the medium into the vessel of choice. Low adhesion pipette tips and tubes are recommended

3. Before removing the cap from the syringe, gently move the plunger back and forth to release it

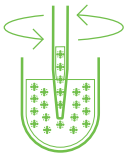
4. Dispense the hydrogel to the medium

5. Swirl the hydrogel mixture gently with the pipette tip

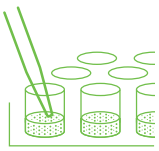
6. Gently pipette the hydrogel mixture up and down for a minimum of 90 seconds and try to avoid air bubbles



7. Detach the cells and prepare the cell suspension. Add the cells slowly and mix gently to avoid the air bubbles from forming



8. Dispense the hydrogel-cell mixture to the wells



9. Carefully add or exchange the medium on the top of the hydrogel-cell mixture against the wall of the well

$$\text{Volume of stock} = \frac{\text{Final volume of assay x required hydrogel concentration (\%)}}{\text{Hydrogel stock concentration (\%)}}$$

Volume of culture media = Final volume of assay – Volume of stock hydrogel – Volume of cell suspension

6.1. Which growth factors to start with?

GrowDex hydrogel works as a blank canvas for you to customize cell culture conditions as you require, using media and growth factors. Growth factors can be added in media which is then mixed with GrowDex. During media changes, this growth factor supplemented media can also be used. This allows you to establish reproducible assays with consistent results. As animal-derived matrices contain undefined growth factors which also vary in concentration, you might want to try to have a similar, but controlled, growth factor environment when optimizing the transfer from an animal-derived to an animal-free matrix. It is also important to note that based on the literature, it is required to add growth factors also to the animal-derived matrices to achieve desired cell behaviour. When working with animal-derived matrices, it is though impossible to precisely control the amount of different growth factors, as the matrix composition and growth factor quantities vary. See below table 3 detailing the suggested concentrations of growth factors at known concentrations based on the literature that could be used as a starting point to optimize the culture conditions.

Table 3: Growth Factors for Cell Culture

Growth factor	Matrigel matrix	Suggested concentrations in cell culture*
IGF-1	5.0 - 24.0 ng/ml	1.0 ng/ml [10, 11]
TGF-b	2.0 - 5.0 ng/ml	0.5 – 10 ng/ml [12-16]
EGF	0.5 - 1.3 ng/ml	10.0 – 100 ng/ml [17-21]
PDGF	5.0 - 48.0 pg/ml	0.01 – 1.0 ng/ml [22, 23]
VEGF	1.0 - 6.5 ng/ml	25.0 – 100.0 ng/ml [24-26]

*concentrations of growth factors to be added in the medium and these should be adjusted according to the cell type.

References; IGF-1: Mira et. al., (1999) [10]; Samani et. al., (2007) [11]. TGF-b: Hurrell et. al., (2020) [12]; Ogasawara et. al., (2020) [13]; Fujiwara et. al., (2020) [14]; Fan et. al., (2020) [15]; Guerrero-Martínez et. al., (2020) [16]. EGF: Baker et. al., (2020) [17]; Cui et. al., (2018) [18]; Hogrebe et. al., (2020) [19]; Vinckier et. al., (2020) [20]; Dinger et. al., (2020) [21]. PDGF: Fiedler et. al., (2002) [22]; Demoulin and Essaghir et. al., (2014) [23]. VEGF: Weis and Cheresh et. al., (2005) [24]; Esser et. al., (1998) [25]; Chang et. al., (2000) [26].

7. Example protocols for direct transfer from Matrigel to GrowDex

Please see below example protocols for the direct transfer from Matrigel to GrowDex with MCF7 breast cancer cells, iPSC derived neuronal cells, and smooth muscle and endothelial cells. For results and pictures, please follow the links provided with each example.

7.1. Breast cancer MCF7 cells

MCF7 cells were cultured in 3D in GrowDex and in 2.5D on top of Matrigel. Full application note here [5].

Materials

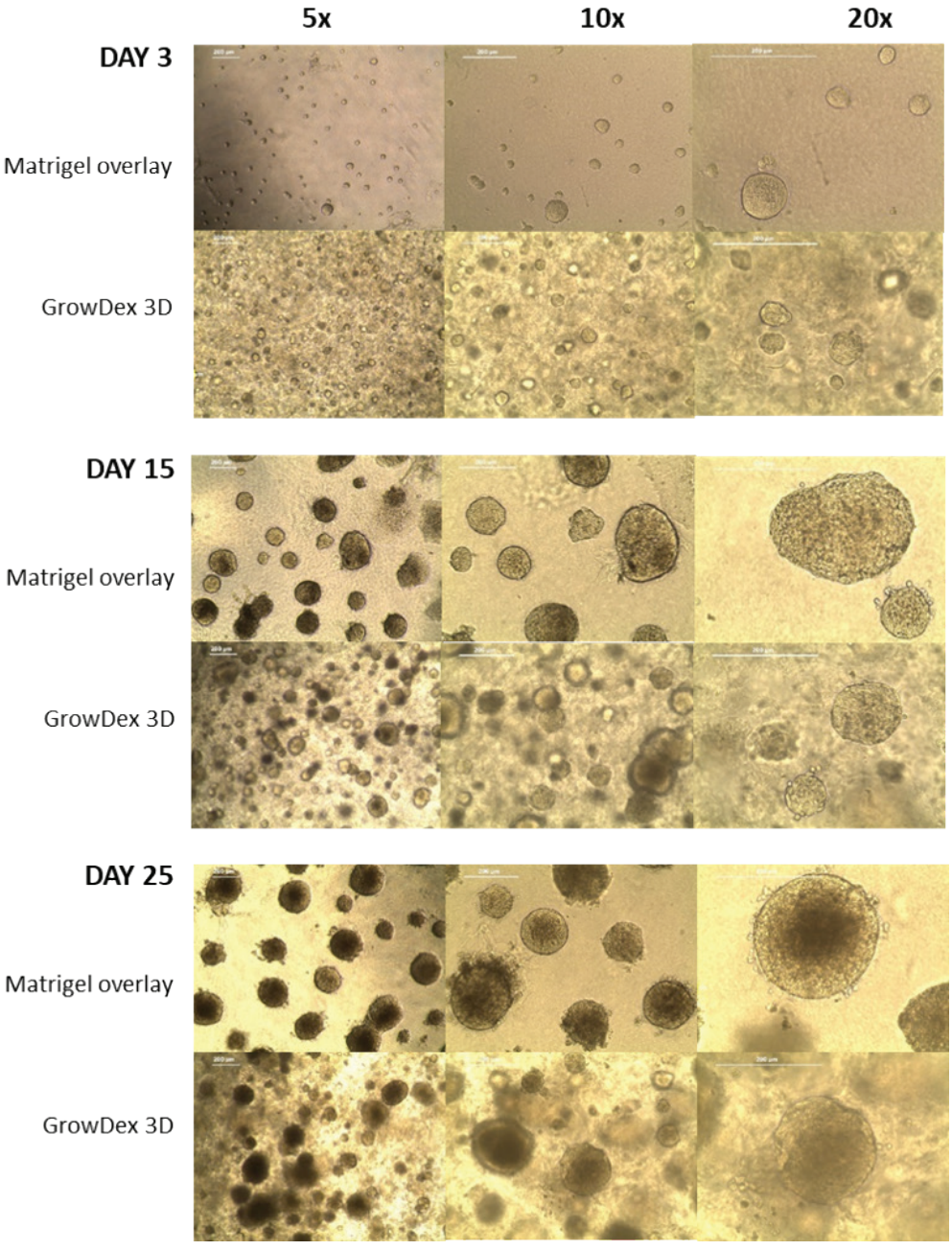
- GrowDex 1.5% (Cat No. 100 103 005, UPM Biomedicals)
- Matrigel GFR (Cat No. 356230, Corning)
- 96-well plates (Cat No. 3370, Corning)
- 8-well chamber slides (Cat No. 177445, Nunc)MCF7 cells
- TrypLe Express (Cat No. 12604039, Thermo Fisher)
- Maintenance and 3D culture medium
  - 89% RPMI 1640 supplemented with 11% FBS

GrowDex

- MCF7 cells were cultured in 0.37% GrowDex concentration
- Cell seeding density was 25K cells / 100ul of GrowDex-medium mixture in a well of 96-well plate
- 130 µL culture medium was added on top of GrowDex
- Medium was replaced every other day, and every day after culture day 10

Matrigel

- 45 µL undiluted Matrigel coating / 8-well chamber slide (pre-hardened with 20min / +37°C incubation)
- 3000 cells were seeded in 300 µL of 2% (v/v) Matrigel –medium mix / well
- 400 µL culture volume for 2% Matrigel–medium


5x
10x
20x
DAY 3
Matrigel overlay
GrowDex 3D
DAY 15
Matrigel overlay
GrowDex 3D
DAY 25
Matrigel overlay
GrowDex 3D

7.2. iPSC derived neuronal cells

Human iPSC derived neural progenitor cells were cultured in 3D embedded in GrowDex and in 2.5D on top of Matrigel. Full application note here [27]. These cultures were similarly performed using the same cells but instead cultured in GrowDex-T. The GrowDex-T application note can be accessed here [28].

Materials

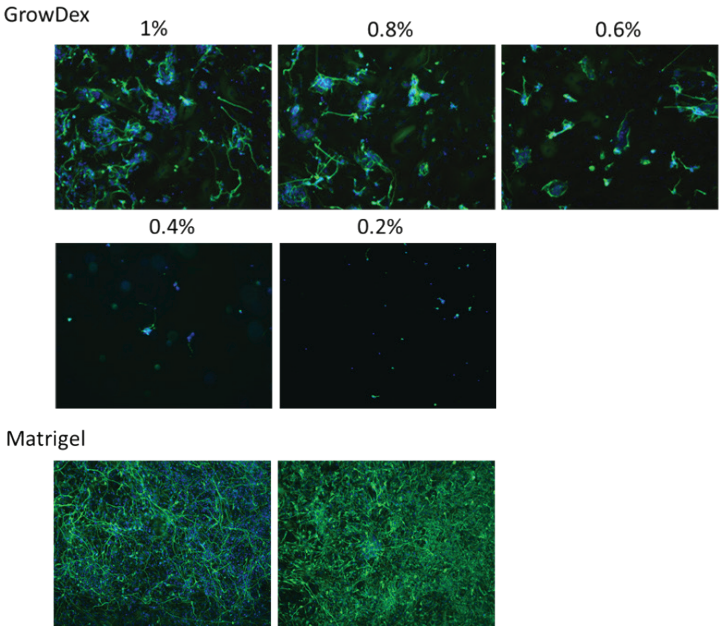
- Human induced pluripotent stem cells derived neural progenitors [29]
- DMEM/F12 media (Cat No. 11330032, ThermoFisher)
- B27 Supplement (Cat No. 17504044, ThermoFisher)
- GrowDex®, 1.5% (Cat No. 100 103 005, UPM)
- Matrigel (Cat No. 354277, Corning)

GrowDex

- GrowDex concentrations were 1, 0.8, 0.6, 0.4 and 0.2 %.
- GrowDex hydrogel volume was 100 µl per well
- Cell concentration for all conditions was 50,000 cells per well
- Cells were fed every other day with DMEM/F12 containing 2% B27. Half of the media was replaced as to not disturb the hydrogel layer

Matrigel

- Matrigel concentration was used at a concentration of 0.08 mg/ml
- Matrigel volume was 100 µl per well.
- Cell concentration was 50,000 cells per well
- The media was aspirated, and the cells were plated on top of the Matrigel layer
- Cells were fed every other day with DMEM/F12 containing 2% B27



7.3. Smooth muscle and endothelial cells

Smooth muscle and endothelial cells were cultured both embedded in and on top of GrowDex or Matrigel. Full application note here [30].

Materials

- Primary human umbilical cord smooth muscle cells (SMC, self-isolated)
- Primary human umbilical cord vein endothelial cells (HUVEC, self-isolated)
- GrowDex 1.5% (Cat No. 100103002, UPM Biomedicals)
- Matrigel (Cat No. 354234, Corning)
- 96-well plate flat bottom (Cat No. 655 180, Greiner Bio-one)
- RPMI 1640 culture media supplemented with 10% FCS
- SMC culture media (Cat No. 200 0601, Provitro)
- EGM-2 media (Cat No. 200 0101, Provitro)

Preparation of GrowDex

GrowDex was diluted in a round bottom tube with RPMI 1640 media to a working concentration of 0.5%. Mixing was done by slowly pipetting up and down to achieve a homogenous solution without air bubbles.

Preparation of Matrigel

Matrigel was left to thaw overnight in the fridge on ice. Also 96-well plate and pipette tips were cooled before use. Matrigel was diluted 1:6 with cold RPMI 1640 media and immediately put back on ice.

Cell seeding on top of matrix

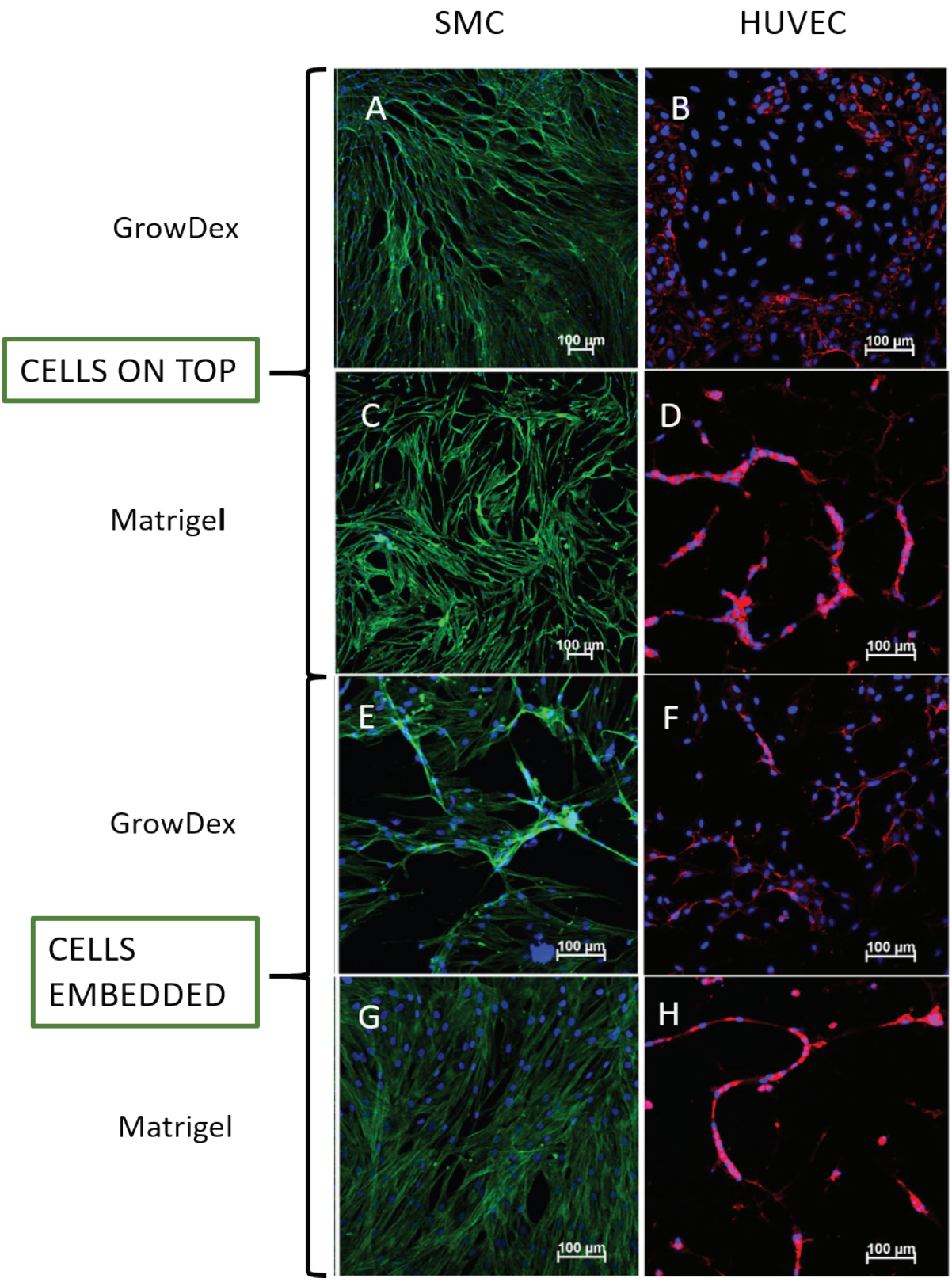
- 40 µL matrix was used per well on 96-well plate.
- Before adding 40 µL of matrix (GrowDex or Matrigel) per well, the wells were washed 2 times with RPMI 1640. The plate was placed in the incubator for 30 min before adding the cells.
- After 30 minutes, 10 000 cells per well in 200 µL culture media were seeded on top of the matrix and incubated at +37°C for 7 days.

Cell seeding embedded in matrix

- 100 µL matrix was used per well on 96-well plate.
- 10 µL cell suspension with 10 000 cells in culture media was prepared and mixed with 90 µL diluted matrix (GrowDex or Matrigel) per well.
- Cells embedded in matrix were dispensed into the well and put in the incubator for 30 min, 100µL culture media was added on top, and the plate was incubated at +37°C for 7 days.

Media change

Culture media was changed after 3 days. 50 µL media was carefully removed and the same volume of fresh media was added.


SMC
HUVEC
GrowDex
CELLS ON TOP
Matrigel
GrowDex
CELLS EMBEDDED
Matrigel
A
B
C
D
E
F
G
H
100 µm

8. References

1. Hughes, C.S., L.M. Postovit, and G.A. Lajoie, Matrigel: A complex protein mixture required for optimal growth of cell culture. PROTEOMICS, 2010. 10(9): p. 1886-1890.
2. Aisenbrey, E.A. and W.L. Murphy, Synthetic alternatives to Matrigel. Nature Reviews Materials, 2020. 5(7): p. 539-551.
3. Patel, R. and A.J. Alahmad, Growth-factor reduced Matrigel source influences stem cell derived brain microvascular endothelial cell barrier properties. Fluids and Barriers of the CNS, 2016. 13(1): p. 6.
4. Talbot, N.C. and T.J. Caperna, Proteome array identification of bioactive soluble proteins/peptides in Matrigel: relevance to stem cell responses. Cytotechnology, 2015. 67(5): p. 873-883.
5. Niklander, J. and L. Paasonen, Breast Cancer Cell Line MCF7 3D Culture Demonstrated in GrowDex® and Matrigel, in UPM Biomedicals Application Note. UPM-Kymmene Oyj, Helsinki, Finland.
6. Vukicevic, S., et al., Identification of multiple active growth factors in basement membrane matrigel suggests caution in interpretation of cellular activity related to extracellular matrix components. Experimental Cell Research, 1992. 202(1): p. 1-8.
7. Bhattacharya, M., et al., Nanofibrillar cellulose hydrogel promotes three-dimensional liver cell culture. Journal of Controlled Release, 2012. 164(3): p. 291-298.
8. Söderholm, S., et al., Best Practices for Pipetting 3D Cell Culture Hydrogels, in UPM Biomedicals Application Note. UPM-Kymmene Oyj, Helsinki, Finland.
9. Mäkelä, R., et al., Ex vivo modelling of drug efficacy in a rare metastatic urachal carcinoma. BMC Cancer, 2020. 20(1): p. 590.
10. Mira, E., et al., Insulin-Like Growth Factor I-Triggered Cell Migration and Invasion Are Mediated by Matrix Metalloproteinase-9*. Endocrinology, 1999. 140(4): p. 1657-1664.
11. Samani, A.A., et al., The Role of the IGF System in Cancer Growth and Metastasis: Overview and Recent Insights. Endocrine Reviews, 2007. 28(1): p. 20-47.
12. Hurrell, T., et al., Human Liver Spheroids as a Model to Study Aetiology and Treatment of Hepatic Fibrosis. Cells, 2020. 9(4): p. 964.
13. Ogasawara, N., et al., Role of RANK-L as a potential inducer of ILC2-mediated type 2 inflammation in chronic rhinosinusitis with nasal polyps. Mucosal Immunology, 2020. 13(1): p. 86-95.
14. Fujiwara, A., et al., Effects of pirfenidone targeting the tumor microenvironment and tumor-stroma interaction as a novel treatment for non-small cell lung cancer. Scientific Reports, 2020. 10(1): p. 10900.
15. Fan, J., et al., Targeting the Notch and TGF-β signaling pathways to prevent retinal fibrosis *in vitro* and *in vivo*. Theranostics, 2020. 10(18): p. 7956-7973.
16. Guerrero-Martínez, J.A., et al., TGFβpromotes widespread enhancer chromatin opening and operates on genomic regulatory domains. Nature Communications, 2020. 11(1): p. 6196.
17. Baker, M.J., et al., Evaluation of active Rac1 levels in cancer cells: A case of misleading conclusions from immunofluorescence analysis. Journal of Biological Chemistry, 2020. 295(40): p. 13698-13710.
18. Cui, Y.-H., et al., FBXL14 abolishes breast cancer progression by targeting CDCP1 for proteasomal degradation. Oncogene, 2018. 37(43): p. 5794-5809.
19. Hogrebe, N.J., et al., Targeting the cytoskeleton to direct pancreatic differentiation of human pluripotent stem cells. Nature Biotechnology, 2020. 38(4): p. 460-470.
20. Vinckier, N.K., et al., LSD1-mediated enhancer silencing attenuates retinoic acid signalling during pancreatic endocrine cell development. Nature Communications, 2020. 11(1): p. 2082.
21. Dinger, T.F., et al., Microenvironmentally-driven Plasticity of CD44 isoform expression determines Engraftment and Stem-like Phenotype in CRC cell lines. Theranostics, 2020. 10(17): p. 7599-7621.
22. Fiedler, J., et al., BMP-2, BMP-4, and PDGF-bb stimulate chemotactic migration of primary human mesenchymal progenitor cells. Journal of Cellular Biochemistry, 2002. 87(3): p. 305-312.
23. Demoulin, J.-B. and A. Essaghir, PDGF receptor signaling networks in normal and cancer cells. Cytokine & Growth Factor Reviews, 2014. 25(3): p. 273-283.
24. Weis, S.M. and D.A. Cheresh, Pathophysiological consequences of VEGF-induced vascular permeability. Nature, 2005. 437(7058): p. 497-504.

25. Esser, S., et al., Vascular Endothelial Growth Factor Induces Endothelial Fenestrations In Vitro. Journal of Cell Biology, 1998. 140(4): p. 947-959.
26. Chang, Y.S., et al., Effect of Vascular Endothelial Growth Factor on Cultured Endothelial Cell Monolayer Transport Properties. Microvascular Research, 2000. 59(2): p. 265-277.
27. Schwab, A., GrowDex® Supports Human iPSC-derived Neural Progenitor Differentiation in vitro, in UPM Biomedicals Application Note. UPM-Kymmene Oyj, Helsinki, Finland.
28. Schwab, A., GrowDex®-T Supports Human iPSC-derived Neural Progenitor Differentiation in vitro in UPM Biomedicals Application Note. UPM-Kymmene Oyj, Helsinki, Finland.
29. Ebert, A.D., et al., EZ spheres: A stable and expandable culture system for the generation of pre-rosette multipotent stem cells from human ESCs and iPSCs. Stem Cell Research, 2013. 10(3): p. 417-427.
30. Mimler, T., Smooth muscle cells and endothelial cells: Comparative study of 3D culture in GrowDex® & Matrigel™, in UPM Biomedicals Application Note. UPM-Kymmene Oyj, Helsinki, Finland.
31. Mikkonen, P., UPM Biomedicals

GrowDex®

www.growdex.com

To order products online: www.perkinelmer.com
To request a quote or place an order: www.perkinelmer.com/contactus
For technical support: Global.TechSupport@perkinelmer.com

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

PerkinElmer®
For the Better

An Authorized Distributor