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3D Cell Culture Tools and Applications

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Lab & Production Materials

Advancing Life Science

We are a leading science and technology company in healthcare, life science and performance materials. Around 50,000 employees work globally to continually develop and enhance technologies that improve and enhance life – from biopharmaceutical therapies to treat disease, cutting-edge systems for scientific research and production, to liquid crystals for smartphones and LCD televisions. Founded in 1668, we are the world's oldest pharmaceutical and chemical company.

Our Life Science teams provide scientists and engineers with best-in-class technologies, lab materials and services with the intention of making research and production simpler, faster and more successful. Our solutions enable scientists to spend more time advancing the promise of science through technologies that help detect the previously undetectable.

Pioneering 3D Cell Culture Technologies

Today, we are pioneering innovations in three-dimensional (3D) cell culture and leading the industry with a comprehensive and highly published portfolio of state-of-the-art 3D cell technologies including tumor spheroids, stem cell organoids, and tissue engineering via 3D bioprinting.

We are pleased to introduce the first commercially available human organoids, including on-demand colon organoids, a lung organoid culture system and through a partnership with the University of Michigan, a biobank of patient-derived gastrointestinal organoids—all with specialized media, reagents and a range of tools, protocols and support for 3D research.

Cover: Immunocytochemical characterization of human iPS cell-derived colon organoids positive for α-carbonic anhydrase-IV/DAPI. (Page 13)

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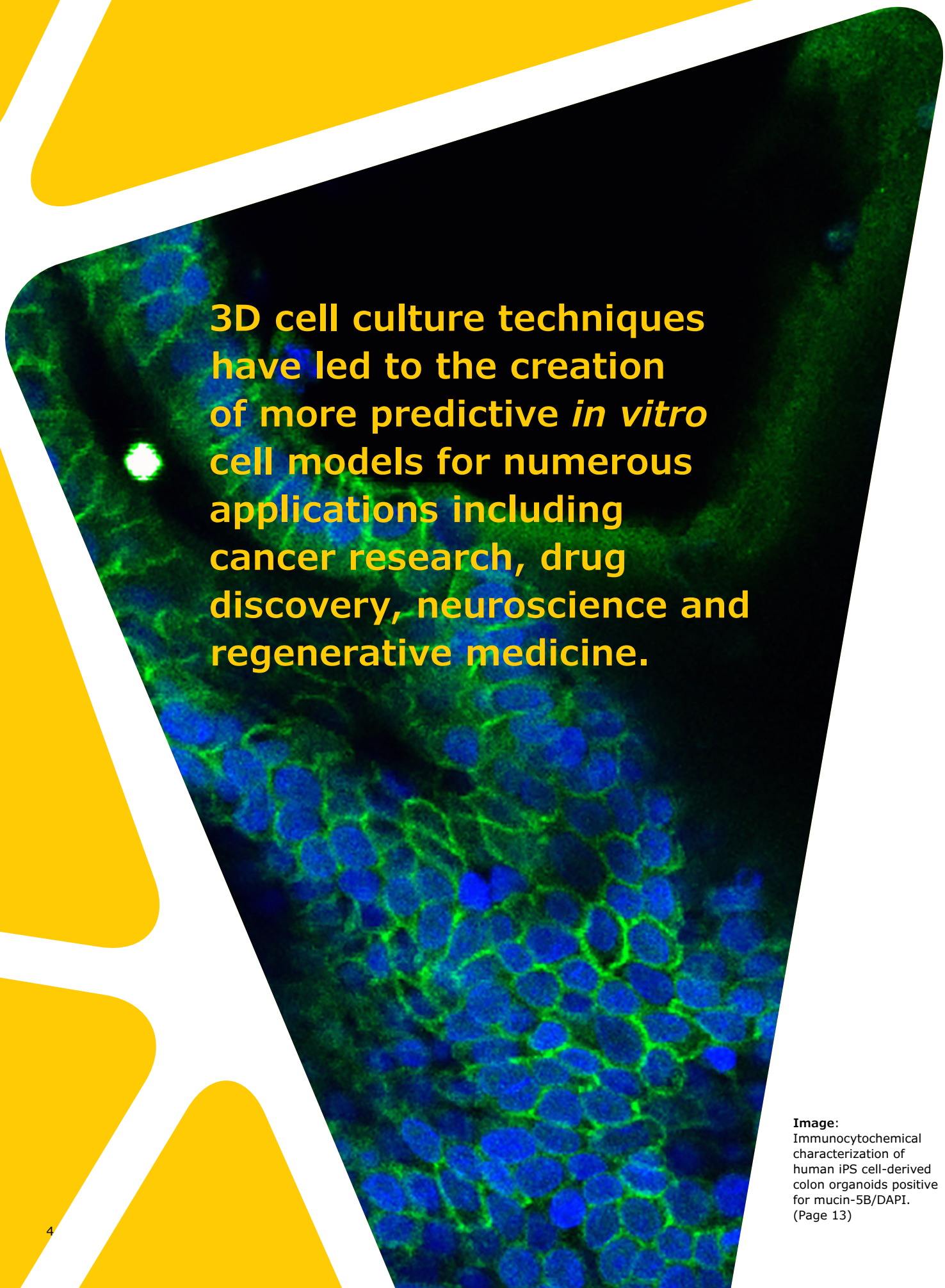
3D Cell Culture Tools and Applications

Cells naturally grow and differentiate in 3D environments. Recently, the use of advanced 3D cell culture approaches like tumor spheroids, stem cell organoids and tissue engineering via 3D bioprinting have been implemented to more closely model *in vivo* cellular responses. Improved 3D cell culture models can accurately replicate the natural tissue environment to provide more meaningful scientific conclusions and advance human health.

Image: Branching and alveolar lung organoids developed using the 3dGRO™ Lung Organoid Culture System. (Page 16)

Lung bud organoids express the ACE2 receptor for SARS-CoV-2 and the serine protease TMPRSS2, entry point for SARS-CoV-2 infection and viral replication.



3D cell culture techniques have led to the creation of more predictive *in vitro* cell models for numerous applications including cancer research, drug discovery, neuroscience and regenerative medicine.

Image: Immunocytochemical characterization of human iPS cell-derived colon organoids positive for mucin-5B/DAPI. (Page 13)

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3D Cell Culture

Cells in their natural environment have constant interactions with extracellular matrix (ECM) proteins and other cells, regulated by complex biological functions like cellular migration, apoptosis, or receptor expression. In traditional two-dimensional (2D) cell cultures, most of these interactions are lost or significantly reduced.

Three-dimensional (3D) cell culture systems allow cells to grow or interact with their surroundings in all three dimensions, simulating the *in vivo* physiological microenvironment.

3D cell culture techniques have led to the creation of more predictive *in vitro* cell models in diverse disciplines that include cancer research, drug discovery, neuroscience and regenerative medicine.

Advanced 3D cell systems allow researchers to bridge the gap between classical 2D cell culture and *in vivo* animal models.

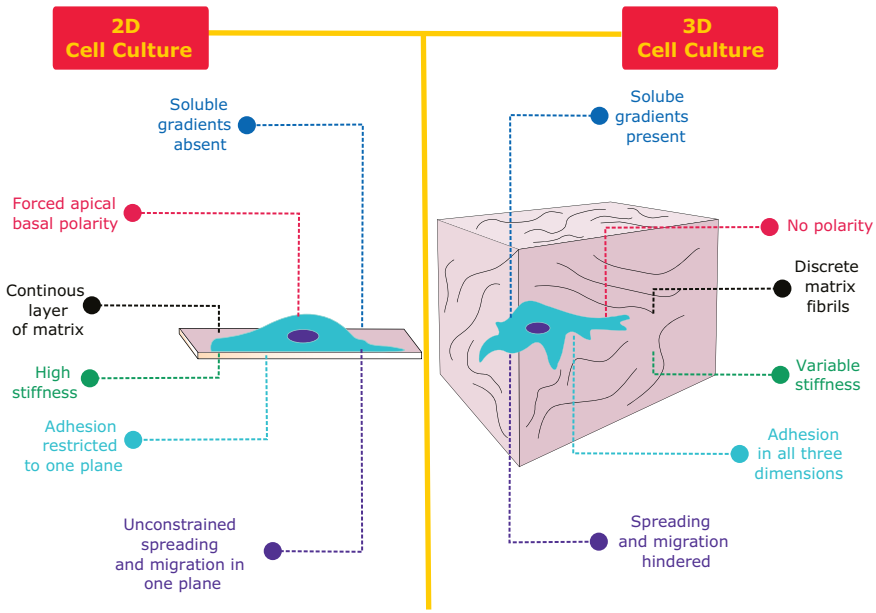


Figure 1. 2D vs. 3D cell culture. Primary differences between cell behaviors and constraints when cultivated in 2D compared to cells cultivated in a 3D environment.

2D vs 3D Cell Culture Characteristics

Key Characteristics	2D Cell Culture	3D Cell Culture	Pubmed Reference (PMID)
Cell Shape	Flat and stretched	Natural shape (ellipsoid/polarized) is retained	257683383
Cell-Media Interface	Equal exposure to media components	Increased exposure in upper layer (heterogeneous exposure) Media components are similar to physiological conditions	159758244, 235011055
Cell Junction	Less prevalent and does not resemble physiological conditions	Prevalent and can enable cell to cell communication	226622786
Cell Differentiation	Moderate to poor	Well-differentiated	230223967
Drug Metabolism	Drug metabolism not well observed	Enhanced drug metabolism with increased expression of CYP enzymes	237285278, 167716489
Drug Sensitivity	High efficacy Cells are sensitive	Cells often show resistance and drugs show low potency	1771142310
Cell Proliferation	Higher proliferation rate than in natural environment	Proliferation rate may be high or low, depending on cell type and methods	920151111, 1557619212
Response to Stimuli	Poor response to mechanical stimuli	Well-established responses to mechanical stimuli	2688769813
Viability	Sensitive to cytotoxin	Greater viability Less susceptible to external factors	1771142310
Apoptosis	Highly susceptible to drug-induced apoptosis	Enhanced resistance to drug-induced apoptotic stimuli	1902072914

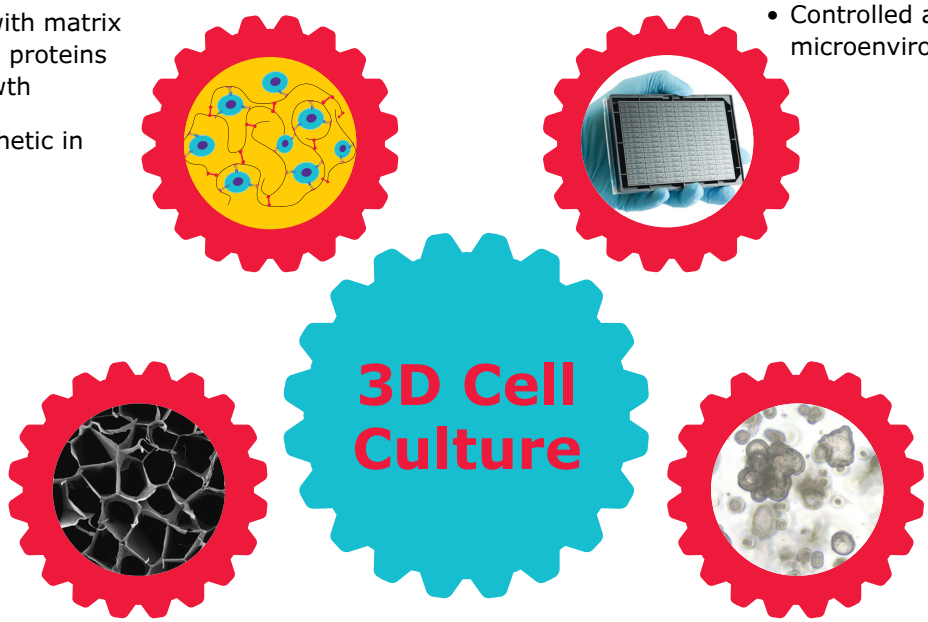
3D cell culture methods may be scaffold-based, using animal derived or synthetic hydrogels and structural 3D scaffolds, or scaffold-free, using free-floating cell aggregates or spheroids. New technologies including organs-on-a-chip devices and stem cell derived organoids can provide more complex tissues with organ-like functionality and phenotypes. 3D cell culture models can be used to elevate the research environment and maximize experimental results.

Hydrogels

- Provides cells with matrix of peptides and proteins to support growth
- Natural or synthetic in origin

Organs-on-a-Chip

- Small scale 3D models
- Controlled and optimized microenvironments



Structural Scaffolds

- Provides cells with structure for growth
- Non-biological polymers

Organoids

- Higher order assembly with *in vivo*-like functionality and phenotypes
- Contain multiple cell types not achievable in 2D or spheroids models
- Derived from adult patient tissues or induced pluripotent stem cells (iPSCs)

Cell Aggregates

- Spheroids form on non-binding surfaces
- Promotes cell growth and adhesion in suspension
- Nutrient gradient achieved with hypoxic necrotic core

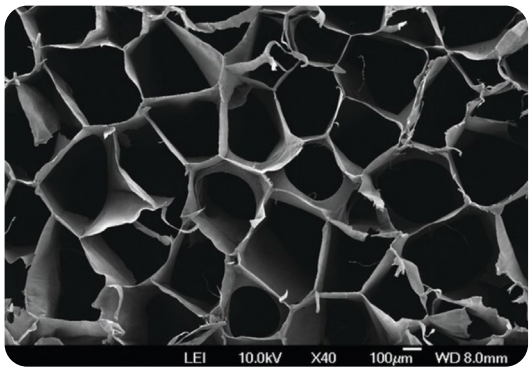
Scaffold-Based 3D Cell Culture Technologies

Structural Scaffolds

3D scaffolds offer the advantage of providing structural support for cell attachment and tissue development. Traditionally made of polymeric biomaterials, scaffolds allow recapitulation of the ECM, the immediate tissue environment of cells. Scaffolds provide attachment sites, supporting cell growth in 3D, and—for some— rigidity for the environment and associated soluble factors (growth factors, cytokines).

Diverse scaffold approaches have been developed by innovative laboratories around the world, and a selection of these technologies are listed below.

Learn More:
SigmaAldrich.com/3Dscaffolds



Product	Overview
SpongeCol® Scaffolds	Ready-to-use sponge composed of highly purified Type I collagen, with an interpenetrating columnar porous architecture structure that permits cells and nutrients complete flow through pores and provides increased surface area for cell attachment, growth and migration
3D Biotek 3D Insert™ PCL Scaffolds	Biodegradable scaffold material for tissue engineering applications made with clear polycaprolactone (PCL), a biodegradable polymer that has been used in the fabrication of surgical implants
3D Biotek 3D Insert™ PS Scaffolds	Transparent scaffolds with porous structure design, made with clear polystyrene (PS) allows researchers to monitor cell growth under an inverted light microscope without the need for specialized equipment
Cellusponge Scaffolds	Natural scaffold made of porous hydroxypropyl cellulose (HPC), good for cryopreservation, available as uncoated, collagen and galactose coated formats to meet all cell culture requirements
Lena Biosciences GradientEZ™ and SeedEZ™ Systems	GradientEZ™ systems enable modeling of gradient-dependent pathological conditions in 3D including cancer, inflammatory and autoimmune diseases, and testing of therapeutic strategies in these models *in vitro*. The SeedEZ™ absorbent 3D cell culture substrate provides a native tissue-like 3D cell environment, and *in vivo*-like round cell morphology
Nanofiber Inserts	Nanofiber cell culture scaffolds support higher cell expansion rates, improved differentiation control, using a synthetic surface with no animal contamination
Millicell® Cell Culture Inserts	Hydrophilic PTFE, PET, polycarbonate and mixed cellulose esters inserts for cell culture with various pore sizes used to support cell growth and nutrient access from both apical and basolateral sides to create organotypic 3D cell models

Hydrogels Overview

3D Hydrogels

Hydrogels are hydrophilic biomaterials that support cellular processes by mimicking the biological environment and encapsulating cells. These water-distended networks of polymers can support cell-adhesion and protein sequestration, using elements of native ECMs. Cells are encapsulated after mixing a cell solution with hydrogel before gel formation, and dispensed in a cell culture vessel during the gelation process. Cells cultured in 3D and embedded in gels recover properties that are more characteristic of cells in their natural environment when compared to traditional 2D cell culture.

How do hydrogels support more physiological cell culture?

- Allow cells to grow in a more physiological 3D structure than traditional, flat 2D cell cultures
- Can be customized to better mimic the natural environment with ECM proteins
- Stiffness/rigidity of the environment can be adjusted to appropriately support the cells of origin
- No specialized protocols, materials or devices needed to adapt cultures to hydrogels

Hydrogel Selection Guide

The first generation of hydrogels used for 3D cell culture were comprised of ECM components generated from crude protein extractions of basement membranes from Engelbreth-Holm-Swarm (EHS) murine sarcoma cells (ECM-based hydrogels). New generations of synthetic, hybrid or peptide-based materials have since been developed to meet specific requirements, including customized environments for cell and tissue types.

	ECM Based Hydrogels (ECM gel, ECM gel EHS, BME, MaxGel™ECM, PureCol® EZ)	Natural Biomimetic Hydrogels (Hystem®)	Synthetic Hydrogels (TrueGel3D™)
Part Number	E1270, E6909, CC131, E0282, 5074	HYS020, HYSC020, HYSHP020	TRUE1-9, TRUEENZ
Biological Relevance	+++	+++	++
Reproducibility	-	++	+++
Easy to use	+	+	++
Potential contaminations	++	-	-
Potential Biological reactivity	+++	++	-
Customization options	-	+	+++
Cell retrieval	-	+	+++
Downstream analysis (Imaging, molecular analysis)	+	++	++
Protocols and application notes available	-	++	+++

TrueGel3D™ Synthetic Hydrogels

Synthetic hydrogels provide increased control, reducing interference that can arise from batch-to-batch variability, contamination and issues with biocompatibility often found in animal-derived hydrogels. TrueGel3D™ synthetic hydrogels do not contain any products of animal origin that could interfere with or contaminate experiments.

TrueGel3D™ synthetic hydrogels are composed of a four-component system, formed by mixing polymers with crosslinkers, cell-interactive components, and user-defined cells. Components included in TrueGel3D™ synthetic hydrogels preserve viability and simulate critical features of the natural ECM. When compared with traditional 2D cell culture conditions, cells in 3D culture encapsulated in hydrogels more closely mimic the cellular environments found in native tissue, supporting cell adhesion and migration. TrueGel3D™ synthetic hydrogel technology provides mechanical and biochemical cues, facilitating investigations in morphological and physiological properties of cells in the 3D environment.

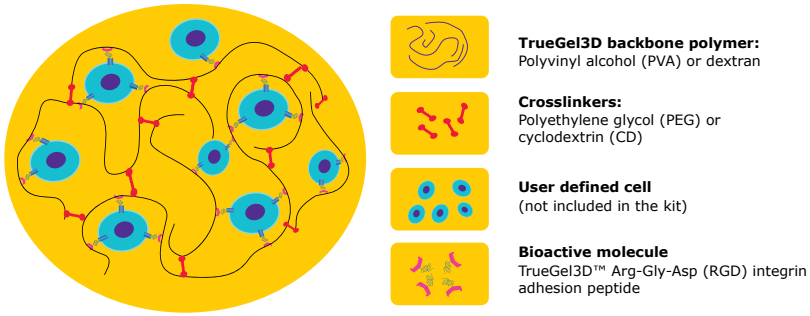


Figure 2. TrueGel3D™ synthetic hydrogels consist of four components: 1) a polyvinyl alcohol (PVA) or dextran backbone polymer, 2) a polyethylene glycol (PEG) or cyclodextrin (CD) thiol-functionalized crosslinker hydrogel, 3) user-defined cells (not included in kits) and 4) cell-interactive components such as Arg-Gly-Asp (RGD) or extracellular matrix proteins (e.g. fibronectin, laminin) that may be added to support adhesion.

Advantages of using TrueGel3D™ synthetic hydrogels to support 3D cultures include:

- Compatibility with a multitude of cells (MDCK, epithelial, fibroblast, cancer, and primary cells including lymphocytes, stromal and embryonic heart muscle cells).
- Easily adjustable gel stiffness.
- Transparency to enhance cell imaging capabilities.
- TrueGel3D™ Kits available with different gel speeds, to support a variety of applications.
- Hydrogel format that provides a non-toxic cell recovery option.
- Defined, animal-free composition to support consistent performance.

Learn More: **SigmaAldrich.com/truegel3D**

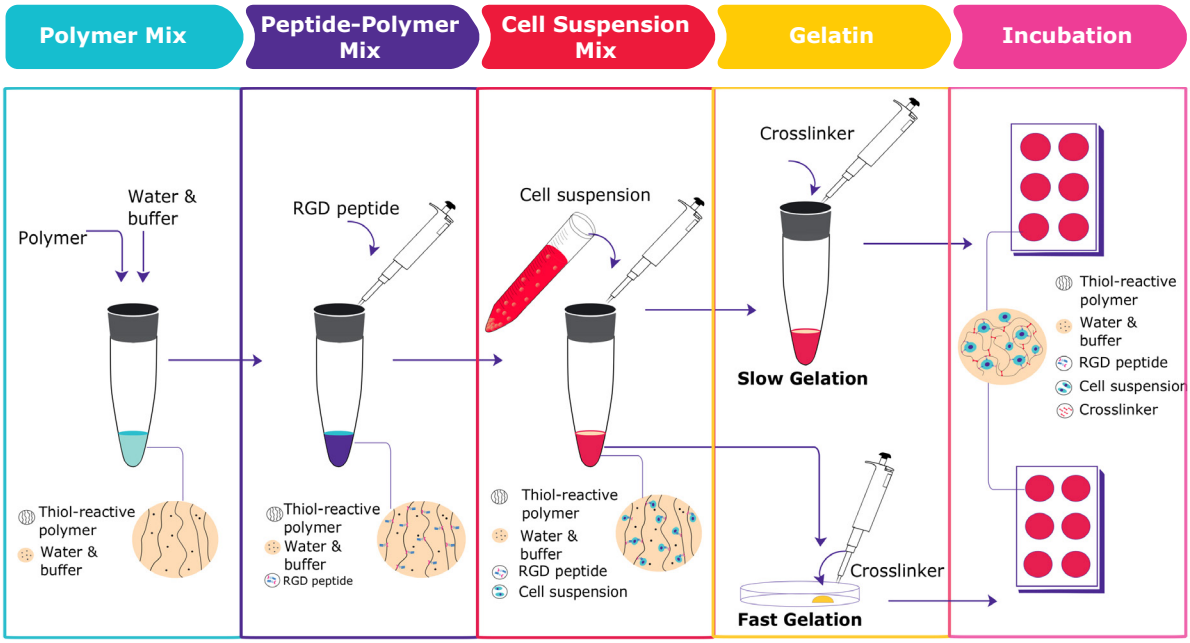


Figure 3. TrueGel3D™ synthetic hydrogel protocol.

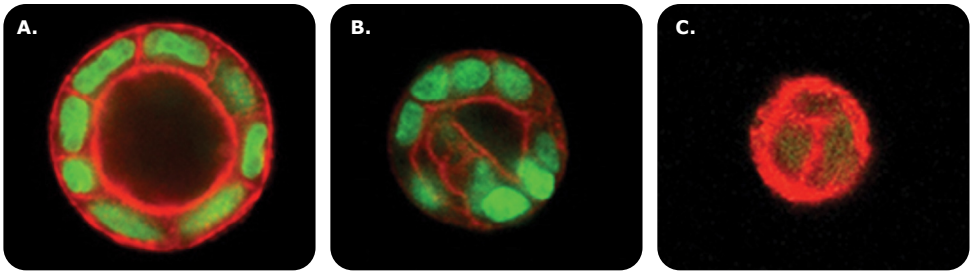


Figure 4. Cyst formation of MDCK cells after 15 days of culture in FAST-PVA TrueGel3D™ synthetic hydrogels modified with (A) 500 µmol/L of RGD integrin adhesion peptide, (B) 500 µmol/L of scrambled RGD peptide and (C) 500 µmol/L of thioglycerol. (Green: nuclei; Red: actin cytoskeleton). A single layer of polarized cells enclosing lumen was observed after 15 days of culture in TrueGel3D™ hydrogels FAST-PVA hydrogel with TrueGel3D™ RGD integrin adhesion peptide. MDCK cysts grown in hydrogels containing TrueGel3D™ scramble RGD integrin adhesion peptide showed lumen but no polarized cells. MDCK colonies grown in thioglycerol-modified hydrogels showed neither lumen nor polarized cells.

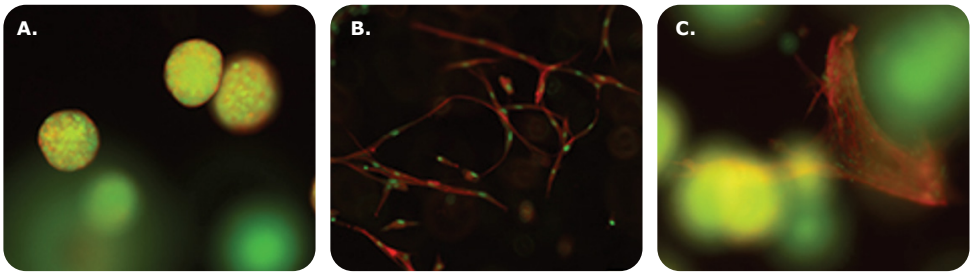


Figure 5. Co-culture of tumor and stromal cells in TrueGel3D™ synthetic hydrogels. (A) MCF-7 breast cancer cell lines formed tumor-like spheroids when cultured alone, (B) human dermal fibroblasts appeared ramified as they would in vivo, and (C) co-culture of both cell types in TrueGel3D™ synthetic hydrogel retained physiological and morphological characteristics even after 14 days, and represented an accurate model to study tumor microenvironments.

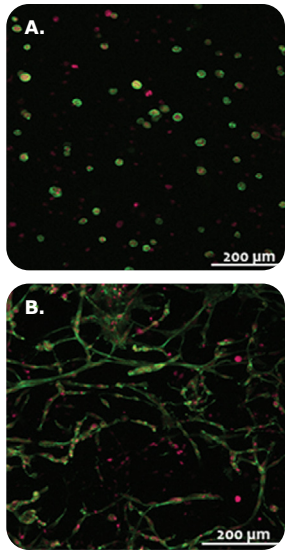


Figure 6. Utilization of TrueGel3D™ synthetic hydrogels to study fibroblast spreading. (A) 3T3 fibroblasts grown in hydrogels with PEG non-cell-degradable crosslinkers appeared round and in tightly packed aggregates, while (B) cells grown in hydrogels with CD-cell degradable crosslinkers spread and migrate throughout the hydrogel.

Product	TRUE1	TRUE2	TRUE3	TRUE4	TRUE5	TRUE6	TRUE7	TRUE8	TRUE9
Backbone Polymer	Dextran	Dextran	Dextran	PVA	PVA	Dextran	Dextran	PVA	PVA
Allows Cell Recovery	✔	✔	✔			✔	✔		
Allows Cell Migration	✔		✔	✔			✔	✔	
Contains RGD Peptide	✔								
Tunable Gel Stiffness						✔	✔	✔	✔
Gelation Rate	Moderate	Fast	Fast	Fast	Fast	Slow	Slow	Slow	Slow

Organoid Culture

Organoids are self-organized 3D cell aggregates derived from primary tissue or from stem cells, are capable of self-renewal and exhibit some organ functionality. Such cultures can be crafted to replicate diverse organ complexities or to express selected attributes or cell phenotypes.

Organoids address the limitations of existing model systems by providing:

- Similar composition and architecture to primary tissue, including self-renewing stem cells, capable of differentiating into cells of all major lineages.
- Biological relevance to model systems, amenable to manipulation of niche components and gene sequences.
- Extended cultivation when cryopreserved for biobanks, or indefinite expansion by leveraging the stem cell capabilities of self-renewal, differentiation and intrinsic ability to self-organize.

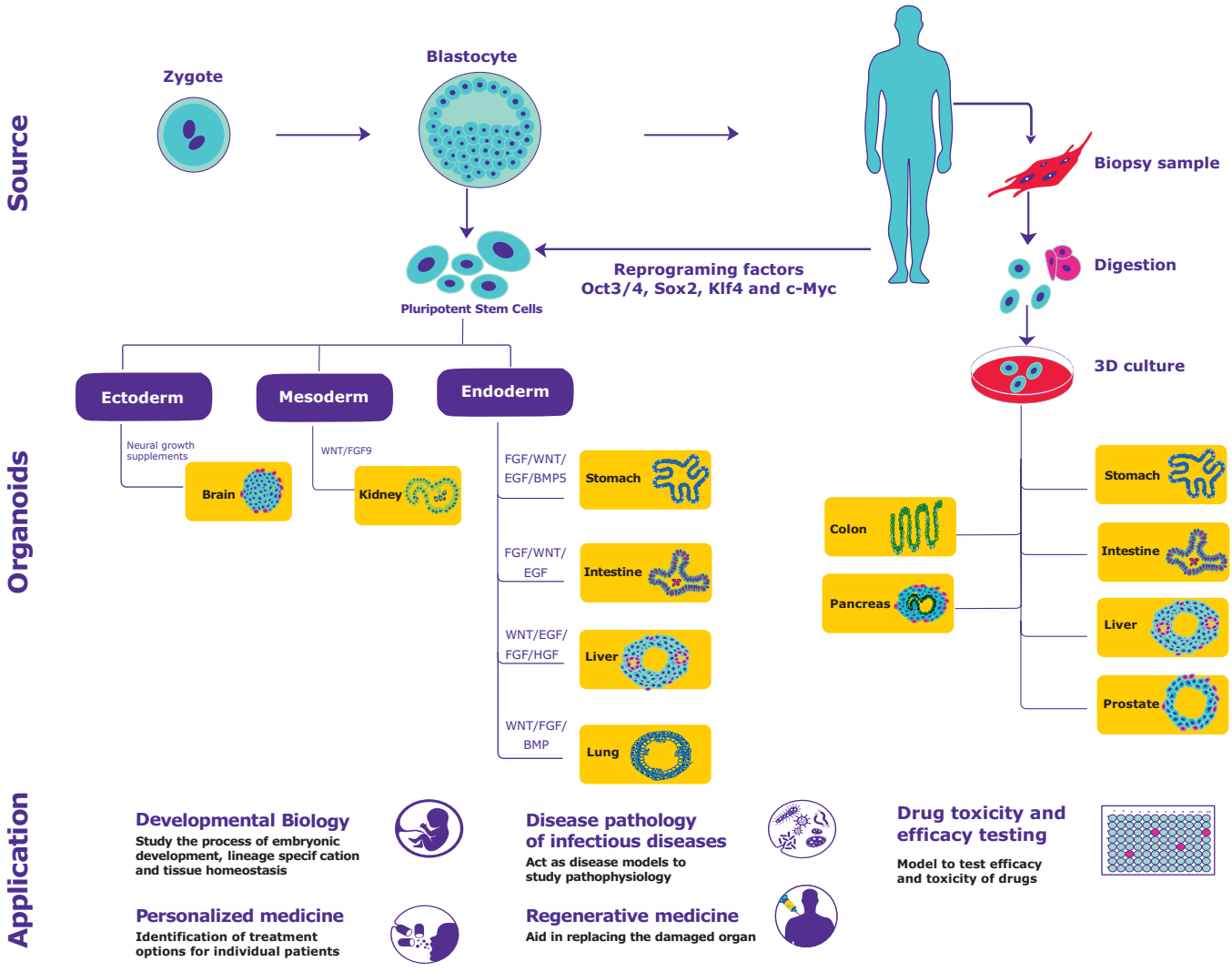


Figure 7. Organoid culture overview. Generation of organoids from primary tissues and pluripotent stem cells enables enhanced relevance in diverse applications.

iPSC-Derived Colon Organoids for Drug Toxicology Screens

Epithelial intestinal organoids—often referred to as enteroids, colonoids or 'mini-guts'—maintain the physiological characteristics of the gastrointestinal system. These useful cell culture models can support studies of intestinal development and diseases such as colon cancer, celiac disease, inflammatory bowel diseases and host-microbiome interactions.

Compared to traditional techniques which rely on lengthy primary tissue isolation, either from mouse or from difficult-to-source human tissue samples, induced pluripotent stem cell (iPSC)-derived organoids can enable the rapid generation of patient-specific cell models from a wider range of human donors. Improved human iPSC-derived colon organoid systems provide highly characterized, assay-ready cryopreserved human colonic organoids and expansion media. Optimized serum-free media and reagents can be used to derive colonic organoids from any human iPS cell line using a simple three-step differentiation process.

Learn More:
SigmaAldrich.com/colonorganoids

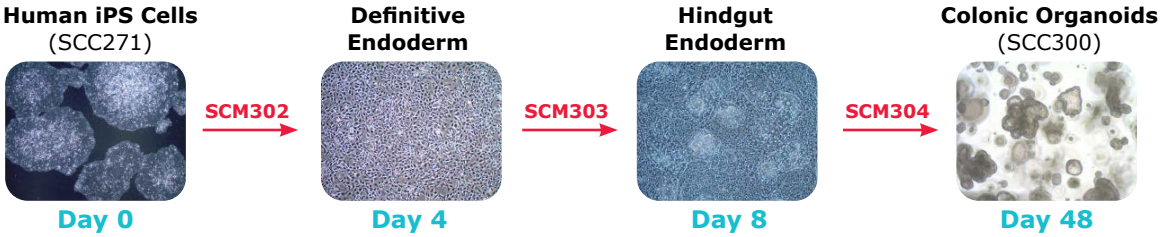


Figure 8. Colon organoid differentiation protocol. Colonic organoids were generated from human iPS cells using a three-step differentiation protocol through definitive endoderm, hindgut endoderm, and colonic organoid expansion stages. SCM302: Definitive Endoderm Induction Medium, SCM303: Hindgut Induction Medium, SCM304: 3dGRO™ Human Colon Organoid Expansion Medium.

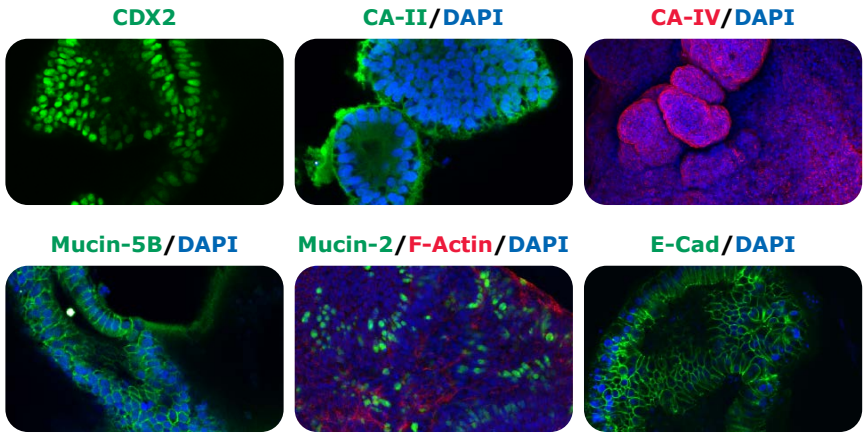


Figure 9. Immunocytochemical characterization of human colon organoids. Human iPS cell-derived colonic organoids are positive for CDX2, α-carbonic anhydrase-II, α-carbonic anhydrase-IV, mucin-5B, mucin-2 and E-cadherin.

iPSC-Derived Colon Organoids for Drug Toxicology Screens (continued)

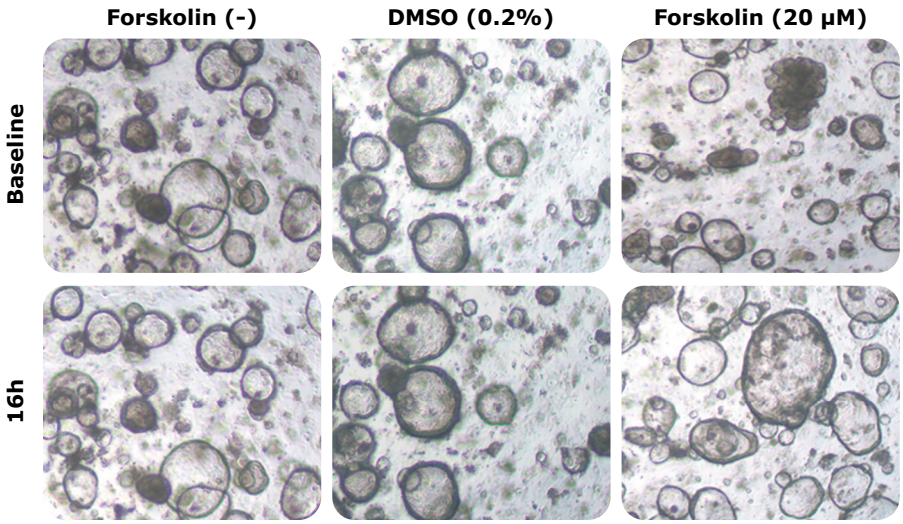
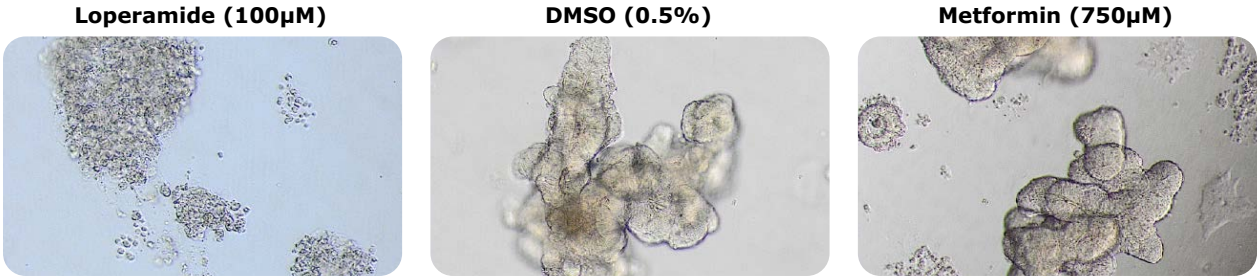


Figure 10. Morphology of colon organoids after forskolin-induced swelling. Wild-type human colon organoids (CFTR+) incubated with forskolin (20 µM) begin to swell at hour three and reach maximal swelling volume at hour sixteen. Colon organoids derived from cystic fibrosis patients (CFTR-) do not swell at any point when subjected to the same conditions.



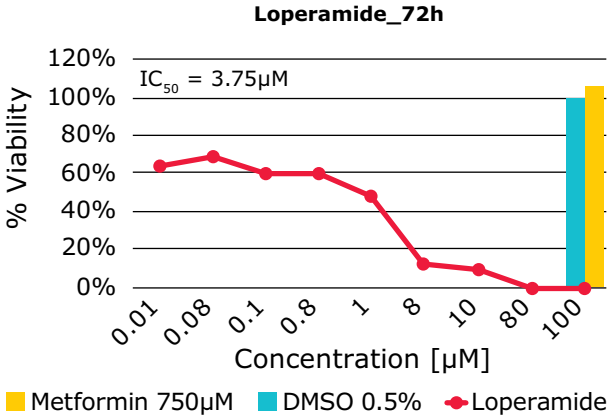


Figure 11. Cytotoxicity testing of compounds for drug screening applications using organoids. Cytotoxic effects of loperamide on human colon organoids measured using the CellTiter-Glo® 3D Cell Viability Reagent. DMSO (0.5%) and metformin (750 µM) used as a vehicle and negative control, respectively.

Ordering Information

Part Number	Description
SCC300	3dGRO™ Human iPSC Derived Colon Organoids
SCM304	3dGRO™ Human Colon Organoid Expansion Medium
SCC271	Simplicon™ Human iPS Cell Line
SCM302	Definitive Endoderm Induction Media
SCM303	Hindgut Induction Medium

Patient Derived Gastrointestinal Organoid Biobank

Patient derived organoids (PDOs) are novel *in vitro* 3D cell models with tissue-like functionality and more clinical relevance than induced pluripotent stem (iPS) cell-derived organoids. Compared to traditional patient-derived xenograft (PDX) and 2D cell models, PDOs and can more closely recapitulate tissue heterogeneity and patient responses to drugs and chemotherapeutics.

We now offer a comprehensive gastrointestinal biobank developed by researchers at the University of Michigan Center for Gastrointestinal Research (UMCGR). These highly characterized tissue-derived human gastrointestinal organoids can be used for disease modeling research.

Gastrointestinal Biobank Overview
The Biobank includes 58 PDO samples, spanning a wide range of demographics (age and sex), with a diversity of tissues (colon, duodenum, ileum, stomach, rectum) and diseases (cancer and ulcerative colitis).

Biobank samples are also:
- Available with RNA-Seq Data
- Low Passage and Highly Viable
- Cultured in Optimized 3dGRO™ L-WRN Conditioned Media

Learn more:
SigmaAldrich.com/intestinalPDObiobank

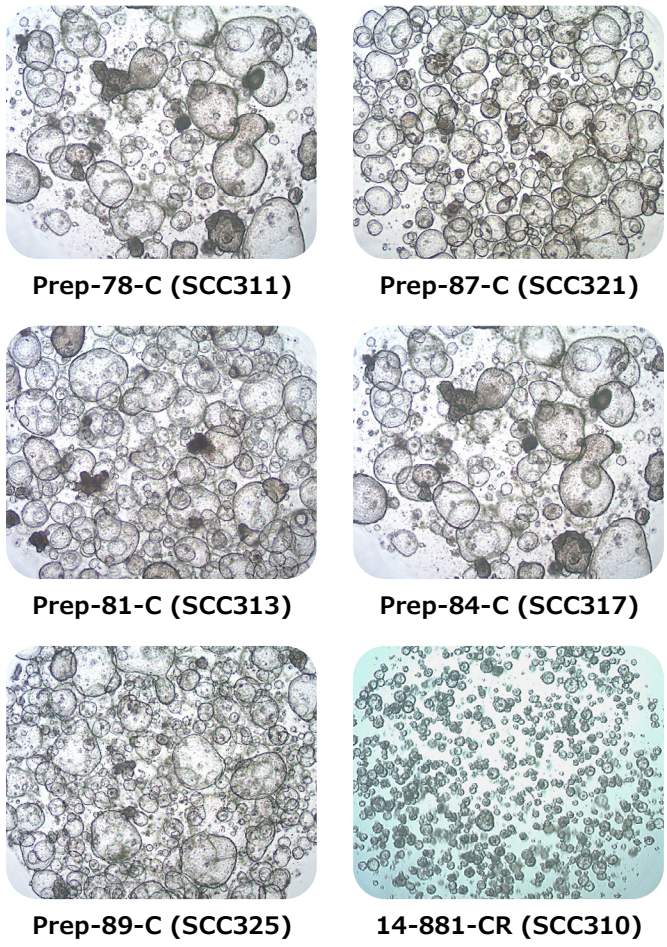


Figure 12. Various morphologies of patient derived colon organoids from both normal (SCC311, SCC321, SCC313, SCC317, SCC325) and diseased patients (colorectal cancer) (SCC310) cultured in L-WRN conditioned media for 5 passages.

Ordering Information

ID	Catalog Number	Age	Sex	Organ	Disease Type or Normal
14881-CR	SCC310	46	M	Colon (Rectum)	Adenocarcinoma (TP53 Pro151Ser, KRAS Gly12Asp, APC Pro1443fs) (RNA-seq)
Prep 78-C	SCC311	52	F	Colon	Normal (RNA-seq)
Prep 80-C	SCC312	29	F	Colon	Normal (RNA-seq)
Prep 81-C	SCC313	49	F	Colon	Normal (RNA-seq)
Prep 81-D	SCC314	49	F	Duodenum	Normal (RNA-seq)
Prep 83-C	SCC315	45	F	Colon	Normal (RNA-seq)
Prep 83-D	SCC316	45	F	Duodenum	Normal (RNA-seq)
Prep 84-C	SCC317	56	F	Colon	Normal (RNA-seq)
Prep 84-D	SCC318	56	F	Duodenum	Normal (RNA-seq)
Prep 85-C	SCC319	62	F	Colon	Normal (RNA-seq)
Prep 85-D	SCC320	62	F	Duodenum	Normal (RNA-seq)
Prep 87-C	SCC321	21	M	Colon	Normal (RNA-seq)
Prep 87-D	SCC322	21	M	Duodenum	Normal (RNA-seq)
Prep 88-C	SCC323	33	F	Colon	Normal (RNA-seq)
Prep 88-D	SCC324	33	F	Duodenum	Normal (RNA-seq)
Prep 89-C	SCC325	55	M	Colon	Normal (RNA-seq)
Prep 89-D	SCC326	55	M	Duodenum	Normal (RNA-seq)

iPSC-Derived Lung Organoids for Respiratory Disease Research

iPSC-derived lung organoids are self-organizing tissues derived from stem cells and serve as useful 3D cell culture models that effectively recapitulate organ morphology and function. Unlike traditional 2D lung airway cell models, which lack the complexity to support a branching alveolar architecture, and *in vivo* animal models, which often lack features of human biology and disease mechanisms, lung organoids provide a unique experimental platform to examine organ development and disease.

Lung organoids are used as models for human respiratory diseases such as cystic fibrosis, asthma, and COPD, as well as for viral infections of the lung including SARS-CoV, H1N1, and MERS. The effects of smoking, vaping and exposure to air pollution on human lung tissues can also be studied with these models.

TECHNOLOGY HIGHLIGHT

The 3dGRO™ Human Lung Organoid Culture System

The 3dGRO™ Human Lung Organoid Culture System is a serum-free, multi-stage culture system for the efficient differentiation of human iPS cells into mature lung organoids that structurally resemble *in vivo* branching airway and early alveolar structures.

The 3dGRO™ system facilitates the production of large numbers of mature lung organoids that express lung and airway cell type markers and enzymes including:

- Surfactant protein B and C
- MUC5AC
- EpCAM, Sox9 and Nkx2.1
- Acetyl-α-Tubulin
- Vimentin
- Angiotensin-converting enzyme 2
- TMPRSS2 serine protease

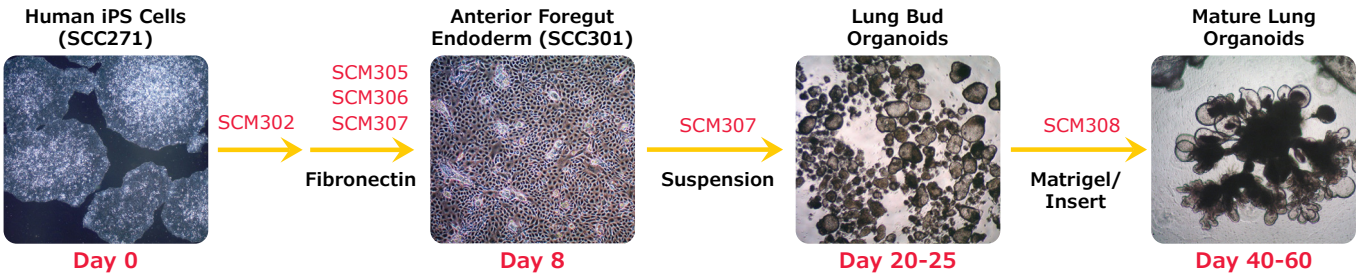


Figure 13. Human pluripotent stem cells were differentiated into definitive endoderm cells using a 4-day induction medium (SCM302). Human definitive endoderm (DE) were directed to anterior foregut endoderm (AFE) with induction media (SCM305, SCM306) during days 4-8. AFE were further differentiated into branching lung bud organoids (LBOs) using the 3dGRO™ Lung Organoid Branching Medium (SCM307) and further matured into branching and alveolar lung organoids using the 3dGRO™ Lung Organoid Maturation Medium (SCM308).

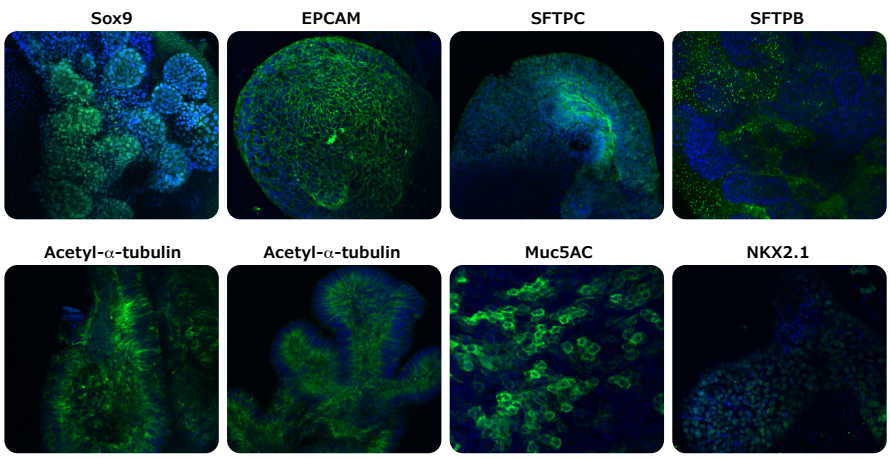


Figure 14. Mature lung organoids derived from human peripheral blood mononuclear cells (PBMCs) and HFF human iPS cells. Pulmonary endoderm (Sox9, EPCAM, NKX2.1), surfactant-producing type II alveolar epithelial cell (SFTPC and SFTPB), ciliated cell (Acetyl-α-Tubulin), and airway goblet cell (Muc5AC) markers are expressed, shown on day 70. Nuclei were counterstained with DAPI.

Lung Organoids and SARS-CoV-2 Research

Lung organoids can be used to study SARS-CoV-2, the virus that causes COVID-19, respiratory viral infections. Mature lung organoids differentiated and matured using the 3dGRO™ system can express the angiotensin-converting enzyme 2 (ACE2) receptor for SARS-CoV-2, along with TMPRSS2, the serine protease that can enhance SARS-CoV-2 viral entry.

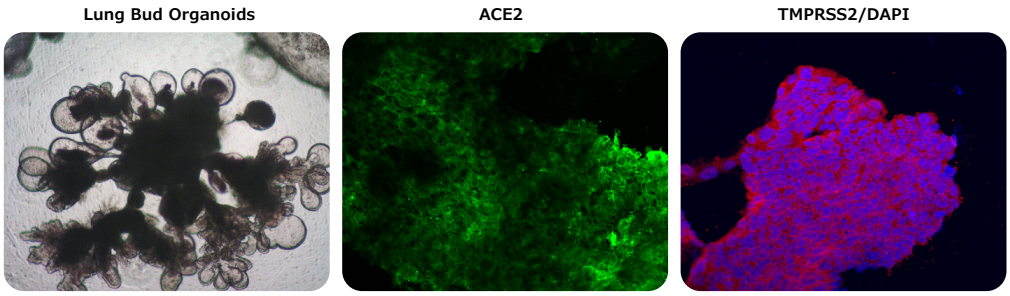


Figure 15. Lung bud organoids express the ACE2 receptor for SARS-CoV-2 and the serine protease TMPRSS2, entry point for SARS-CoV-2 infection and viral replication.

Ordering Information

Part Number	Description
SCC301	Human iPSC Derived AFE Progenitors
SCM302	Definitive Endoderm Induction Media
SCM305	Anterior Foregut Endoderm Induction Medium I
SCM306	Anterior Foregut Endoderm Induction Medium II
SCM307	3dGRO™ Lung Organoid Branching Medium
SCM308	3dGRO™ Lung Organoid Maturation Medium

Learn more: **SigmaAldrich.com/lungorganoids**

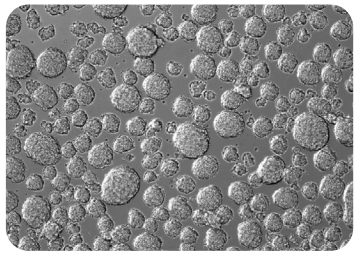
Organoid-Related Reagents

Ordering Information

Part Number	Description
SCM162	DMEM/F-12 PLUS Basal Medium
SCM300	3dGRO™ Organoid Dissociation Reagent
SCM301	3dGRO™ Organoid Freeze Medium
SCC111	R-Spondin1-expressing 293T Cell Line
SCM104	3dGRO™ R-Spondin-1 Conditioned Media Supplement (50 mL)
SCM105	3dGRO™ L-WRN Conditioned Media Supplement (50 mL)
G9020	Gastrin I human
N3376	Nicotinamide
A9165	N-Acetylcysteine
A4403	L-Ascorbic acid
SCM018	Embryoid Body (EB) Formation Medium
E9644	Recombinant Human EGF
SRP4675	Recombinant Human Noggin
SRP3292	Recombinant Human R-Spondin-1 (CHO cells)
SRP6487	Recombinant Human R-Spondin-1 (HEK293 cells)
GF154	Recombinant Mouse Wnt3a
F8924	Recombinant FGF-10
SRP4037	Recombinant FGF-2
S7076	p38 MAP Kinase Inhibitor (SB 202190)
SML1046	CHIR99021 (Glycogen synthase kinase 3 inhibitor)
SML0788	A83-01 (TGFβ kinase/activin receptor-like kinase (ALK 5) inhibitor)
SCM075	ROCK Inhibitor (Y-27632)
HPA012530	Anti-LGR5 polyclonal antibody

Scaffold-Free 3D Cell Culture Technologies

Spheroid Culture Overview



Scaffold-free 3D cell culture techniques allow cells to self-assemble and form non-adherent cell aggregates called spheroids. Spheroids can be derived either from standard cancer cell lines or from primary cells, such as liver hepatocytes.

Spheroids mimic solid tissues by secreting an extracellular matrix and displaying differential nutrient availability and hypoxic gradients that can lead to necrotic hypoxic cores. Spheroids grown using non-scaffold-based techniques are more consistent in size and shape than other 3D models, such as organoids, and provide better *in vitro* cellular models for high-throughput drug screening.

TECHNOLOGY HIGHLIGHT

Spheroid Assays and Analysis

Live/Dead® Cell Viability Assay Kit

The Live/Dead Cell Viability Assay Kit, provides simultaneous fluorescence staining of live and dead cells. Calcein-AM stains live cells green, while Propidium Iodide stains dead cells red, and Hoechst 33342 stains all cells blue for total cell demarcation. The kit is optimized for 3D (spheroid, human organoid and matrix) and 2D cell culture, and can be used for more rapid quantitation of cell viability with flow cytometry.

Advantages of the kit include:

- Versatile applicability for 3D and 2D cell culture and flow cytometry
- Easy-to-use assay protocol
- Intensity of Calcein-AM and Hoechst 33342 stains are stronger compared to other assay kits
- Identification of spatial and temporal patterns of cell death occurring in complex tissues

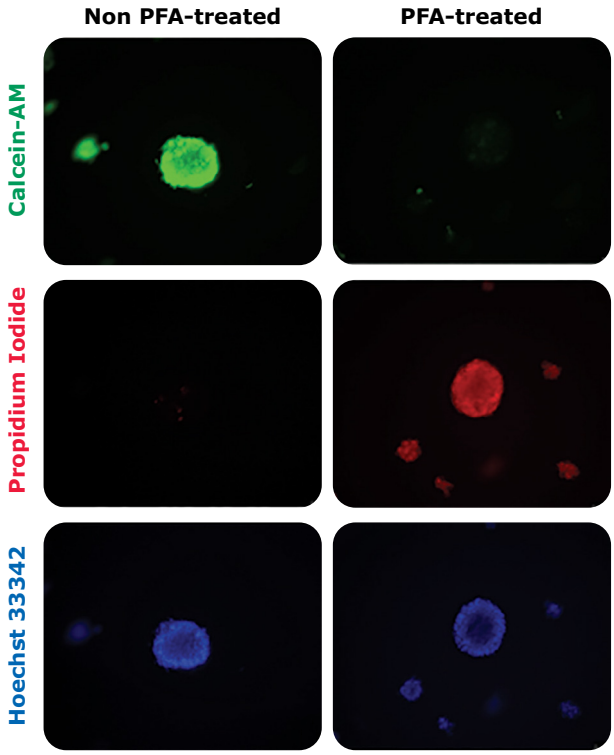


Figure 16. Live/Dead staining of liver spheroids. Day 7 HepG2 spheroids embedded in Matrigel® substrate and stained with the Live/Dead assay before and after selective paraformaldehyde (PFA) fixation/killing.

Ordering Information

Part Number	Description
CBA415	Live/Dead® Cell Viability Assay Kit

Corning® Ultra-Low Attachment (ULA) Spheroid Plates

Ultra-low attachment (ULA) plates enable spheroid maintenance and enhance reproducibility of results from spheroid culture. ULA spheroid plates are hydrophilic and naturally charged to minimize cell attachment, protein absorption, and enzyme activation.

The Corning® ULA spheroid microplate features a novel and proprietary design that offers an all-inclusive culture environment to generate, culture, assay, and analyze spheroids, eliminating the need to manipulate or transfer delicate structures. The microplates have an optically clear, round well-bottom geometry with opaque black walls, and are coated with a hydrophilic, biologically inert and non-degradable Corning® ULA surface. Opaque sidewalls and gridded bottom design reduce well-to-well cross-talk and background fluorescence/luminescence.

The Corning® ULA spheroid microplate is available in 96-, 384-, and 1536-well formats. The 96-well Corning® ULA plate device format is compatible with the HTS Transwell®-96 tissue culture system, used for high-throughput assays in 3D immuno-oncology models.

The Corning® ULA spheroid microplate design facilitates spheroid maintenance and improves reproducibility of results with:

- A covalently bound hydrogel layer that effectively inhibits cellular attachment.
- Surface that minimizes protein absorption, enzyme activation, and cellular activation.
- Non-cytotoxic, biologically inert, and nondegradable surface.
- Non-reversible lids with condensation rings to reduce risk of contamination.
- Individual alphanumerical codes to facilitate well identification.
- Uniform footprint to stack easily.
- Plates sterilized by gamma irradiation and certified nonpyrogenic.

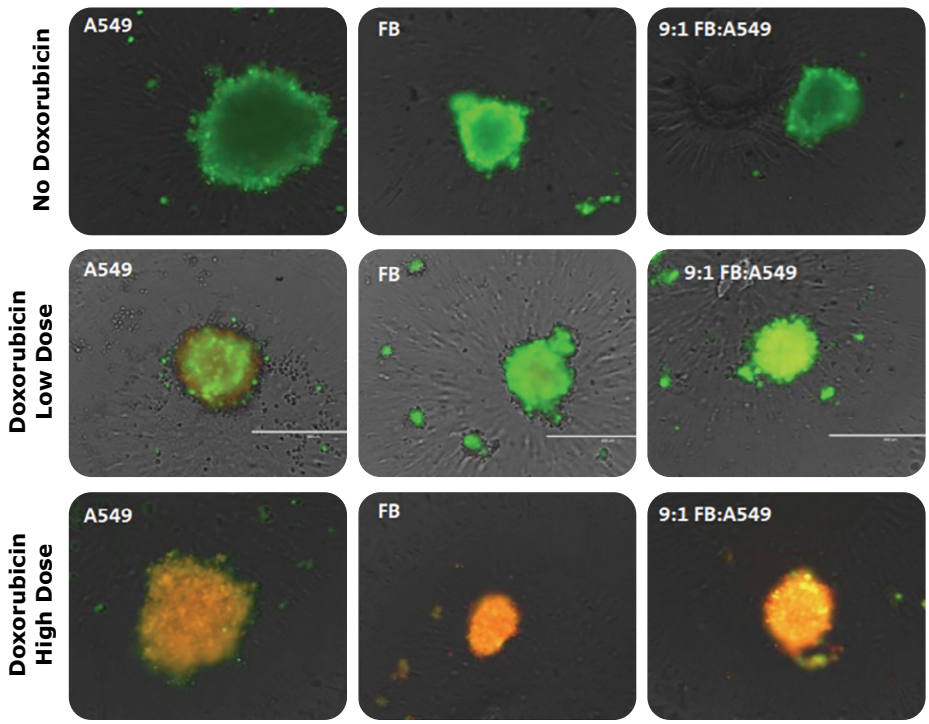


Figure 17. Mono- and co-culture A549 and fibroblast (FB) spheroids were treated with high (34.5 µM) and low (27.6 µM) doses of doxorubicin or vehicle control for 48 hours in Corning® 384-well spheroid microplates. After 96 hours, cell viability and cytotoxicity was assessed with live cells (stained in green) and dead cells (stained in red). FB mono-culture displayed the most intense live staining with low doses doxorubicin, while A549 monoculture showed increased cell death. Low-dose doxorubicin treatments displayed a protective effect in coculture A549 and FB spheroids (9:1 FB:A549).

Ordering Information

Part Number	Description
CLS4515-5EA CLS4520-10EA CLS4520-50EA	Corning® spheroid microplates 96-well, round black/clear bottom, ULA (Ultra-Low Attachment surface), lid, sterile
CLS4516-5EA CLS3830-10EA CLS3830-50EA	Corning® spheroid microplates 384-well, round black/clear bottom, U LA (Ultra-Low Attachment surface), lid, sterile

Visikol® HISTO-M™ Tissue Clearing Reagent

Limitations to visualization and imaging quality of 3D cell cultures can be attributed in part to the use of imaging-based analysis techniques originally developed for 2D cell culture. These techniques can also contribute to sampling bias when imaging is limited to the exterior cells of thick microscopy specimens of spheroids or other 3D structures.

Clearing agents are often used to improve visualization and comprehensive profiling of 3D spheroids. The Visikol® HISTO-M™ tissue optical clearing reagent can reduce spheroid opacity while preserving cellular structures.

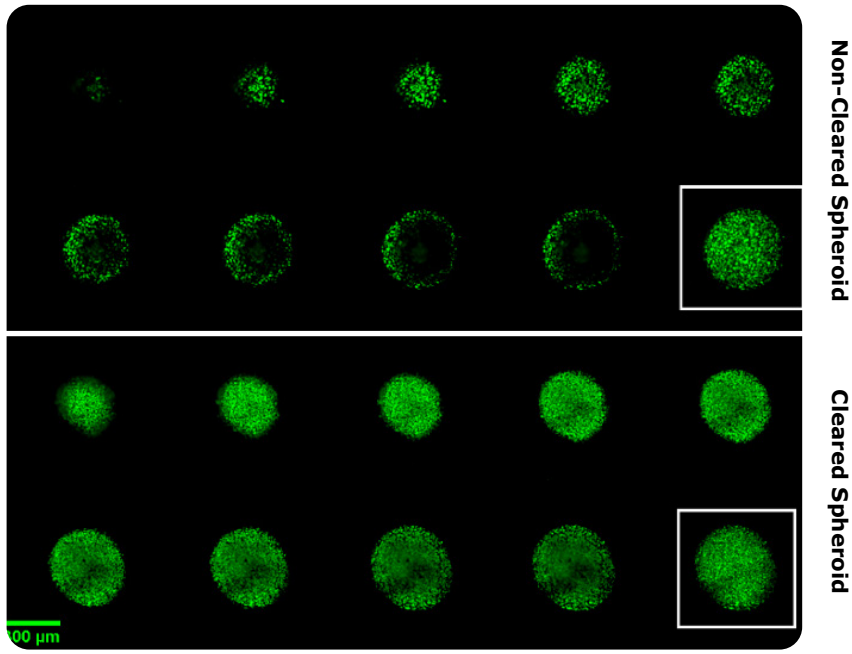


Figure 18. Spheroid opacity is demonstrated in a representative array of slices from confocal image z-stack. Nuclei are stained with SYTOX green. Non-cleared spheroid and cleared spheroid with z-dimension projections (outlined in white) demonstrate improved imaging for interior cells in cleared spheroids. Cell counts for each z-plane are displayed in cleared and non-cleared microtissues (below).

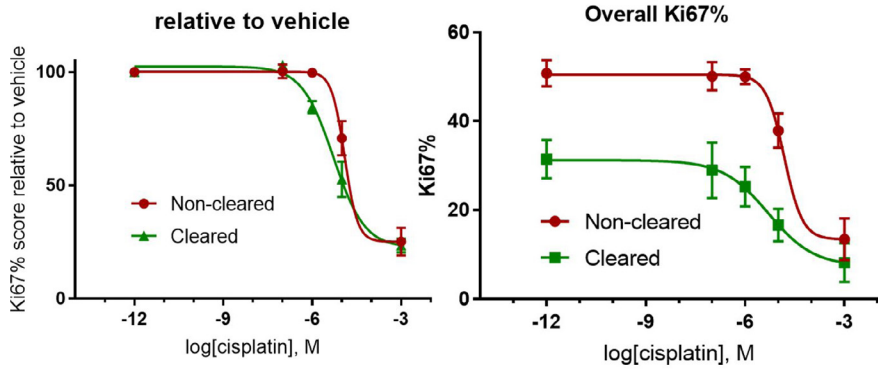


Figure 19. Antiproliferation dose-response curves for cisplatin-treated NCI-H2170 microtissues A) relative to control (vehicle) cell proliferation score; B) showing absolute Ki67 percent proliferation score.

Ordering Information

Part Number	Description
HMSK-1	Visikol® HISTO-M™ Starter Kit
HM-30, HM100	Visikol® HISTO-M™ Tissue Clearing Reagent

The Cancer Stem Cell Hypothesis

Cancer stem cells (CSCs) are minor subpopulations of cancer cells, capable of self-renewal, generating diverse cells in the tumor mass, and sustaining tumorigenesis. CSCs drive tumor progression and recurrence after chemotherapy treatments and have recently become targets for cancer stem cell therapies. CSCs are thought to arise from normal stem cells, progenitor cells, or even more differentiated cells with self-renewing capacity. Since both normal stem cells and cancer cells can self-renew, many pathways that are classically associated with cancer are also involved in the regulation of normal stem cell development. These include Notch, Wnt, Shh, and classical pluripotency transcription factors Oct-4, Sox2, and Nanog.

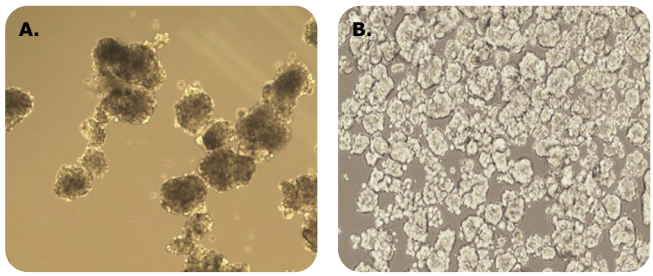


Figure 20. Tumorsphere formation of MCF7 Breast Cancer Cells at Passage 1 (A) and E006AA Prostate Cancer Cells (B) cultured in 3dGRO™ Spheroid Medium.

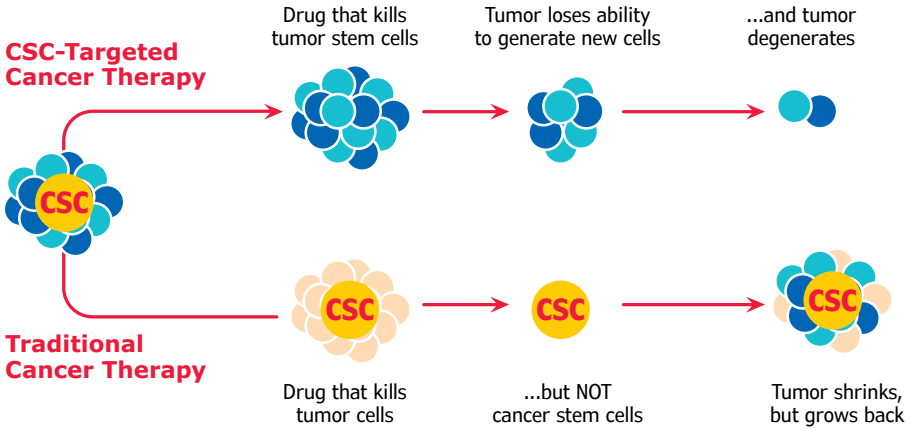


Figure 21. An Illustration of the Cancer Stem Cell Hypothesis.

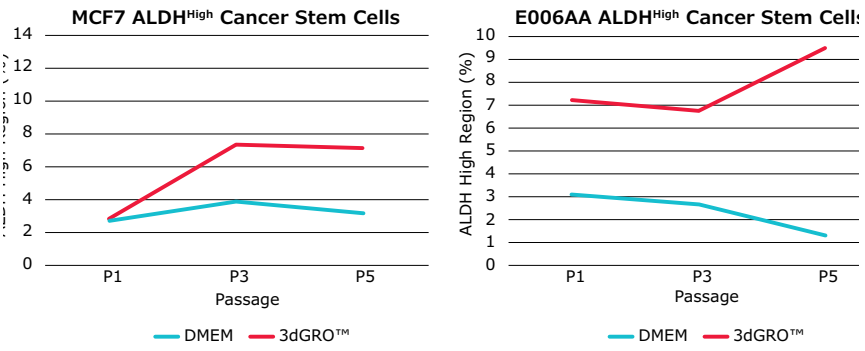


Figure 22. ALDH^{high} stem cell populations of E006AA cells were increased in 3dGRO™ Spheroid Medium at higher passages. ALDH^{high} cancer stem cell populations were enriched when cancer cells were grown in 3dGRO™ Spheroid Medium at passage 5 in 3D tumorsphere cultures. After 3 and 5 passages, ALDH^{high} populations of MCF7 cells were increased to around 8% in 3dGRO™ Spheroid Medium compared to less than 4% of normal (adherent) cultured cells. ALDH^{high} stem cell populations of E006AA cells were increased 7-13% in 3dGRO™ Spheroid Medium after 1, 3, and 5 passages, compared to less than 4% of normal (adherent) cultured cells.

Ordering Information

Part Number	Description
S3077	3dGRO™ Spheroid Medium
SCR150	AldeRed™ ALDH Detection Assay
SCR151	AldeGreen™ ALDH Detection Assay
SCT065	Green-AlDeSense™ ALDH1A1 Live Cell Dye

Emerging Trends in 3D Cell Culture

Organotypic 3D Skin Culture as an *in vitro* Model for the Human Epidermis

Organotypic culture systems (OCSs) support the formation of nearly normal stratified epidermis cells and can mimic complex pathologies of the human skin. This advancement beyond traditional animal models can provide "cruelty-free" *in vitro* organotypic skin models using primary human cells and cell culture inserts.

A step-by-step culture protocol can be used to generate human epidermal skin models. Using primary human keratinocytes (PHK), primary human fibroblasts (PHFs) from dermis, and collagen-coated Millicell® culture inserts, 3dGRO™ Skin Differentiation Medium supports the robust differentiation of keratinocytes during the air-liquid interface culture steps.

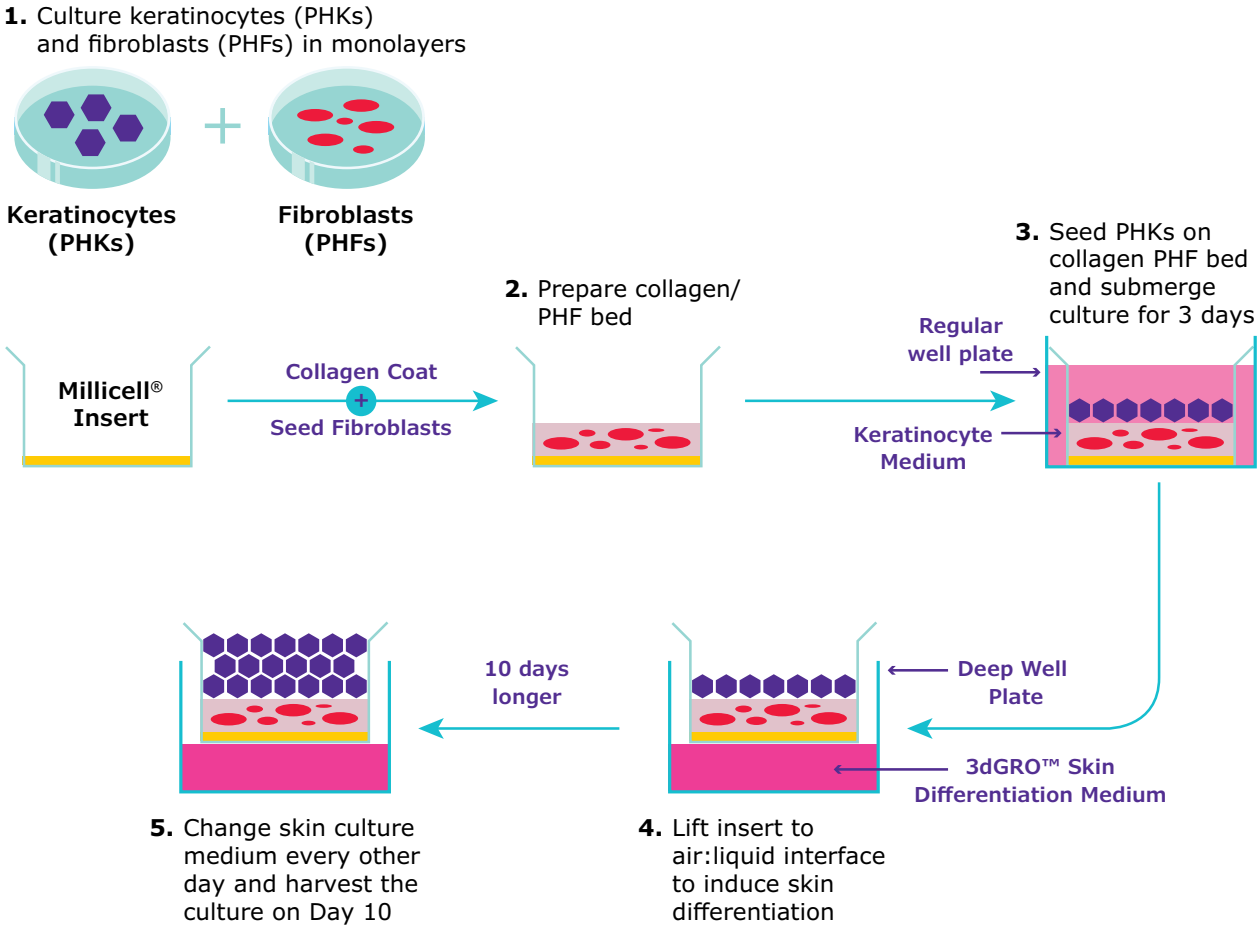


Figure 23. Overview of organotypic human skin culture step-by-step protocol used to generate human epidermal skin models.

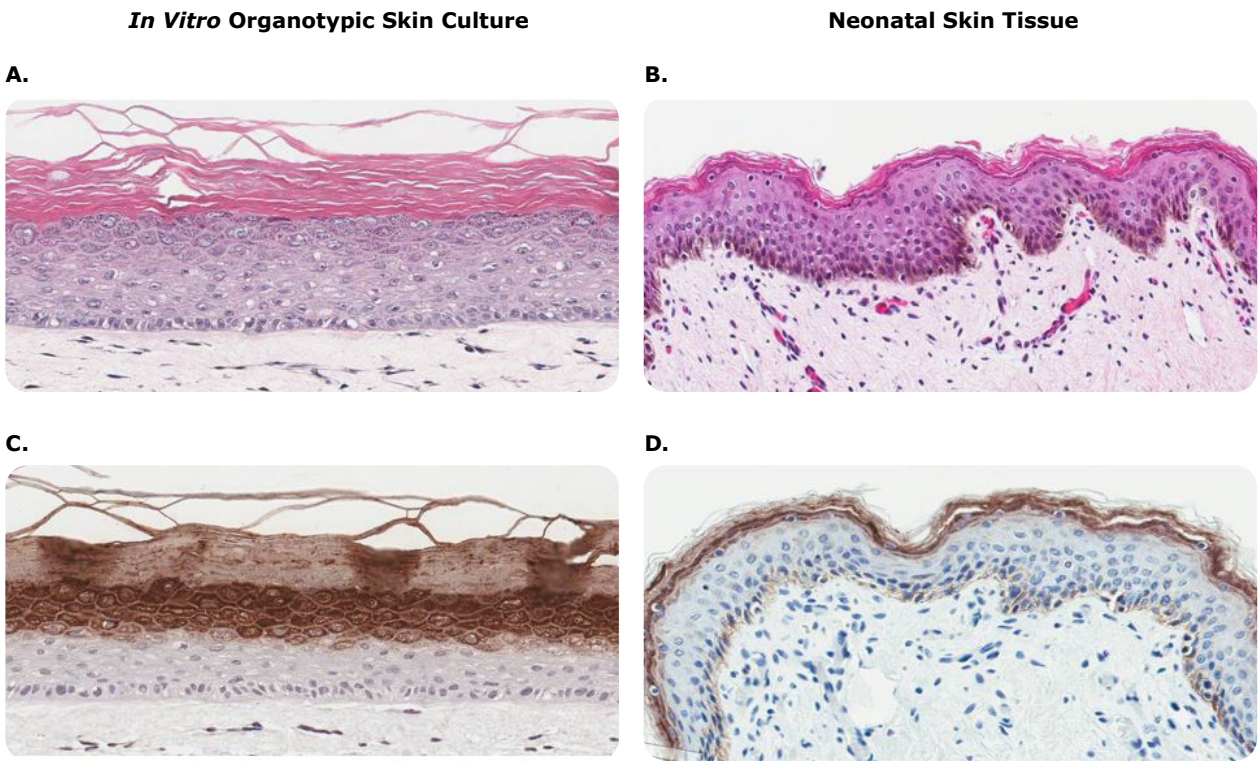


Figure 24. Organotypic skin models have stratified epithelial morphology, comparable to neonatal skin tissue. Hematoxylin and eosin staining of *in vitro* skin culture and *ex vivo* skin sections containing both dermal and epidermal stratified layers (A, B). Filaggrin staining (brown) identifies cornified keratinocyte layers (C, D).

Ordering Information

Part Number	Description
SCM310	3dGRO™ Skin Differentiation Medium
08-115	Collagen, Type I Solution from rat tail
MCHT12H48	Millicell® Hanging Cell Culture Insert, PET 0.4 µm, 12-well, 48/pk
C-12300	Normal Human Dermal Fibroblasts (NHDF) Juvenile Foreskin (PromoCell™)
C-23110	Fibroblast Growth Medium (PromoCell™)
106-05N	Human Dermal Fibroblasts (HDF) Neonatal (Cell Applications®)
116-500	Fibroblast Growth Medium (Cell Applications®)
93061524	Murine NIH 3T3 Cell Line
C-12001	Normal Human Epidermal Keratinocytes (NHEK) Juvenile Foreskin, Single Donor (PromoCell™)
C-12005	Normal Human Epidermal Keratinocytes (NHEK) Juvenile Foreskin, Pooled, (PromoCell™)
C-20111	Keratinocyte Growth Medium 2 (PromoCell™)
102-05N	Human Epidermal Keratinocytes (HEK) Neonatal (Cell Applications®)
131-500	Keratinocyte Serum-Free Growth Medium (Cell Applications®)

3D Neural Stem Cell Models of Alzheimer's Disease

Alzheimer's-In-A-Dish™

Alzheimer's disease (AD) research using *in vitro* human cell models can present challenges in recapitulating the development of toxic amyloid pathology. ReNcell® lines are extensively-published human neural stem cell lines derived from developing human brains. ReNcell® VM and CX cell lines are generated from the ventral mesencephalon and cortical regions of the brain, respectively, and transduced with Myc transcription factors. In addition to the multipotential neuronal and glial differentiation capacity over long-term culture, both cell lines offer phenotype and genotype stability.

3D human neural stem cell models of Alzheimer's Disease have been created using β-amyloid precursor proteins and presenilin-1-overexpressing ReNcell™ VM human neural stem cell lines. This model presents a valuable tool for studying age-related AD dementia, including the production and deposition of amyloid-β and amyloid-β plaques, high levels of phosphorylated tau in the soma and neurites, and the induction of filamentous tau protein.

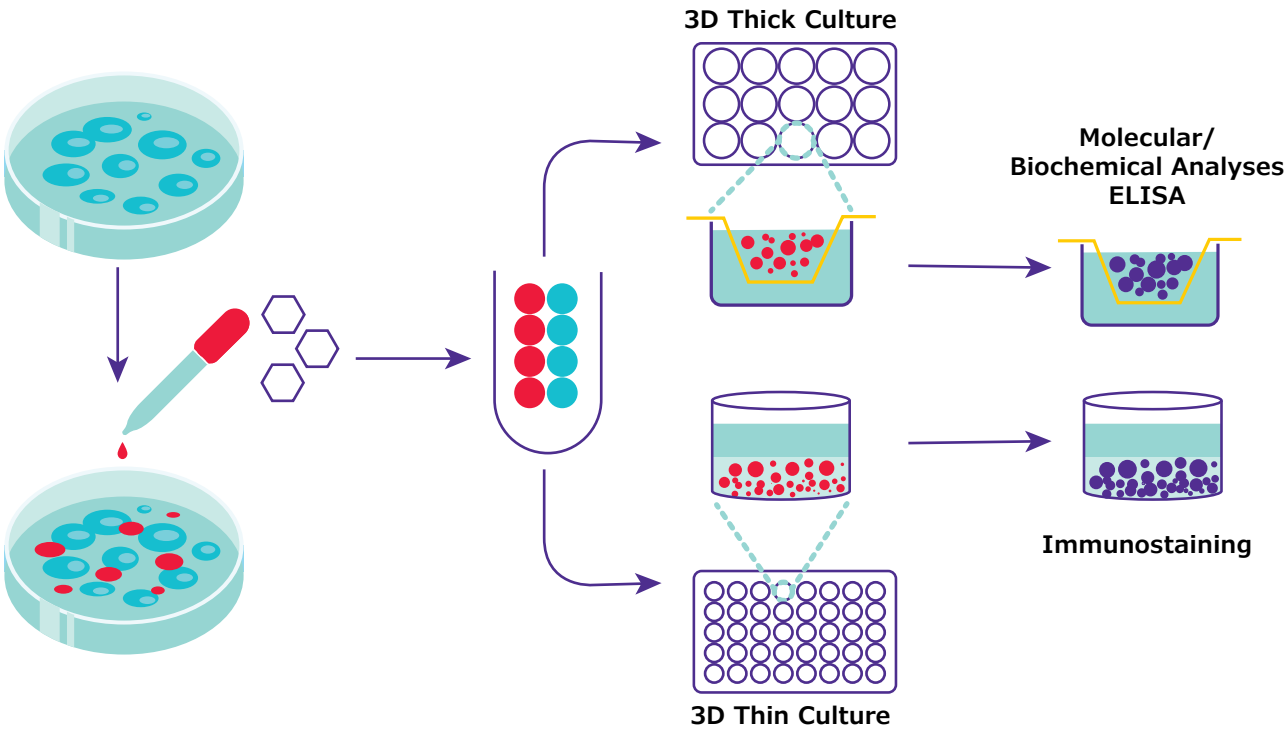
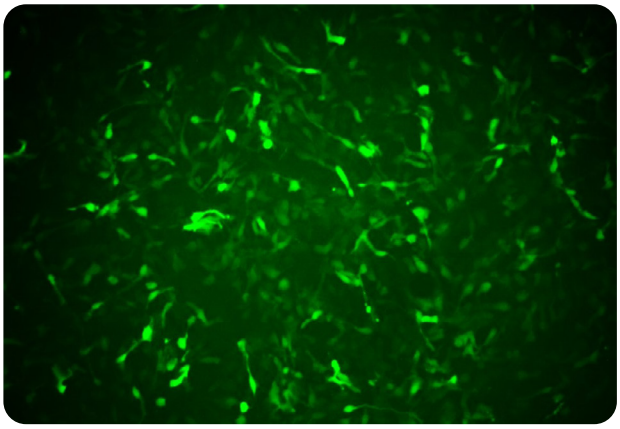


Figure 25. Protocol used to Generate 3D NSC models of Alzheimer's Disease.

A.



B.

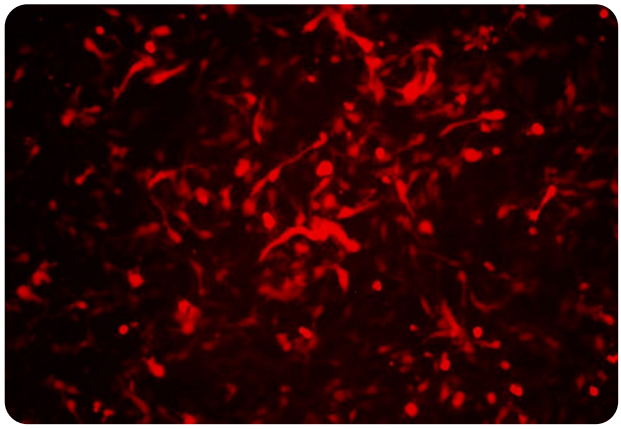
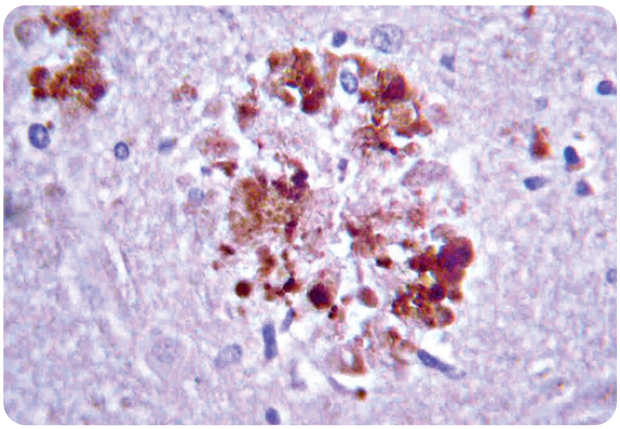


Figure 26. Fluorescence images of ReNcell® VM infected with APPSL-GFP (A) or PSEN1-RFP (B) Lentivirus, MOI =20. Alzheimer's-associated transgenes were expressed after postviral transduction, with >80% transduction efficiency using ReNcell® VM on day 3.

A.



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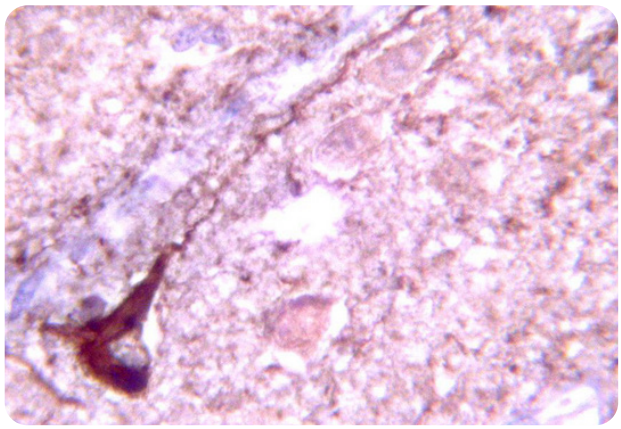

Figure 27. Immunohistochemistry of 3D cultured AD ReNcell® models A) Anti-β-Amyloid antibody (MAB348) staining of AD model. Immunoreactivity is seen as staining on plaque deposits (dark brown). B) Anti-Phospho Tau staining (AB9668) of AD model. Immunoreactivity is clearly not nuclear, and it follows the length of the neuron's axon.

Ordering Information

Part Number	Description
SCC008	ReNcell™ VM Human Neural Progenitor Cell Line
SCC008FAD1	Alzheimer's-In-A-Dish™ ReN-mGAP Clone A4H11 NSC Line
SCC008FAD2	Alzheimer's-In-A-Dish™ ReN-mGAP Clone D4 NSC Line
SCC008FAD3	Alzheimer's-In-A-Dish™ ReN-G2 Clone B2 NSC Line
tableSCC008FAD4	Alzheimer's-In-A-Dish™ ReN-GA2 Clone 3C1 NSC Line
SCC008FAD5	Alzheimer's-In-A-Dish™ ReN-GA2 Clone A5 NSC Line
SCC008FAD6	Alzheimer's-In-A-Dish™ ReN-mAP3 Clone E6F4 P7 NSC Line
SCC008FAD7	Alzheimer's-In-A-Dish™ ReN-mGAP Clone H10 NSC Line
SCM005	ReNcell™ NSC Maintenance Media
SCM007	ReNcell™ Neural Stem Cell Freezing Medium
SCR526	Alzheimer's-In-A-Dish™ APPSL-GFP Lentivirus
SCR527	Alzheimer's-In-A-Dish™ PSEN1-RFP Lentivirus

Human Kidney Nephrotoxicity Models using Organ-on-a-Chip Devices

Organ-on-a-Chip (OOC) devices are micro-engineered cell culture chips lined by human cells, employed to simulate the activity, mechanics and physiological response of entire organ systems. These multi-channel 3D microfluidic cell culture devices grow cells in a manner more relevant and predictive of human *in vivo* conditions.

The proximal tubules carry out diverse regulatory and endocrine functions in the kidney, including reabsorbing salt, water and bicarbonate, and eliminating other substances from the body. In ADME/Tox applications, the proximal tubules are evaluated throughout the drug development process as a target for toxicity. Renal proximal tubule epithelial cells (RPTEC) grown as perfused tubules against an ECM and cultured using a three-lane Mimetas BV OrganoPlate® can form a functional 3D perfused proximal tubule model with advanced renal epithelial characteristics for kidney toxicity and drug screening studies.

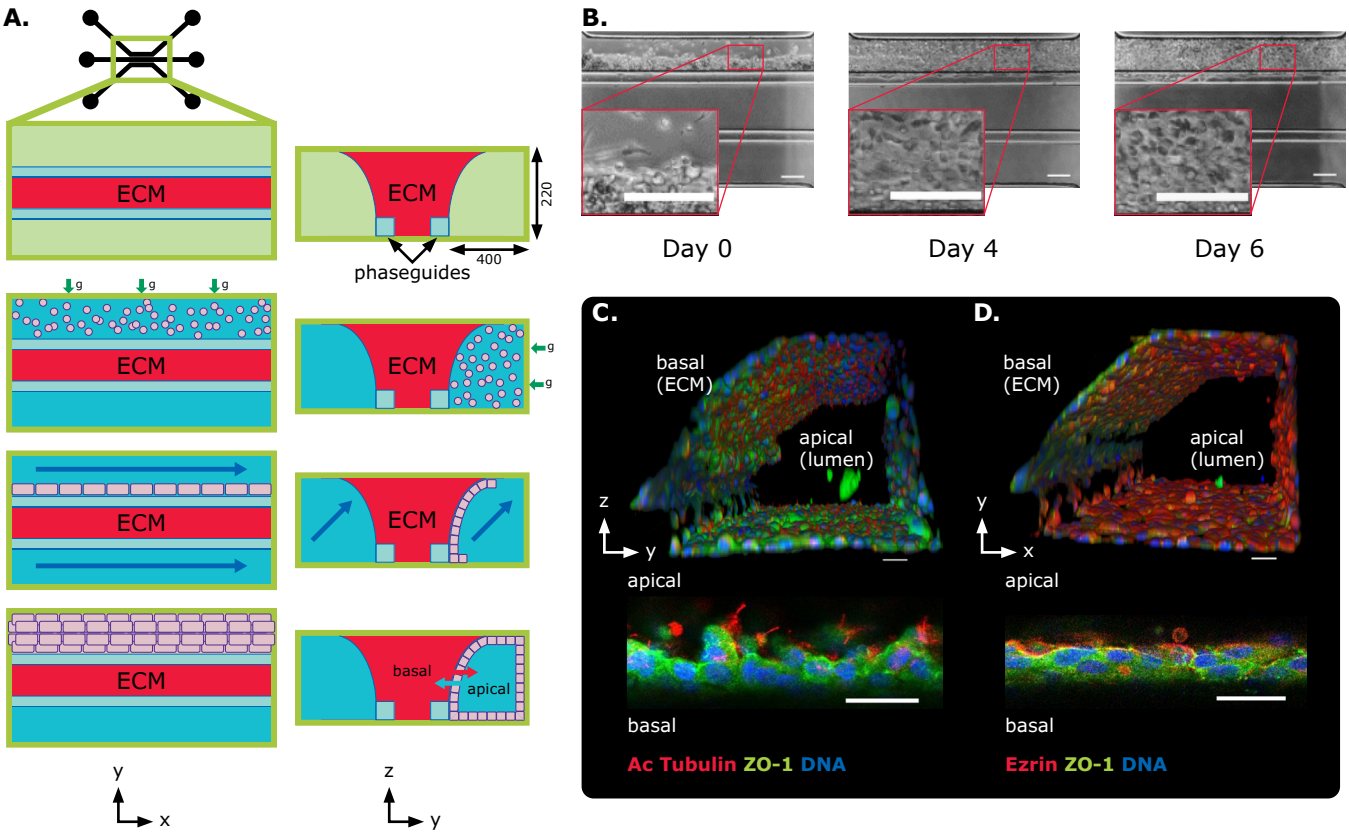

Figure 28. Proximal tubule-on-a-chip model in the 3-lane OrganoPlate® platform. A) SA7K cells were seeded adjacent to ECM collagen I and attached to the gel, growing a perfused tubular structure after gravity-induced attachment. Channel dimensions are given in µm. B) Phase-contrast images of SA7K cells at day 0, 4, and 6 show the formation of a tubular structure in the top channel after six days. Scale bar = 200 µm. C, D) 3D reconstruction images of SA7K tubules in the OrganoPlate® show an apical view into the lumen of tubules. Magnification shows a single z-slice of the cells growing against the ECM. Nuclei in blue. Scale bars = 30 µm.

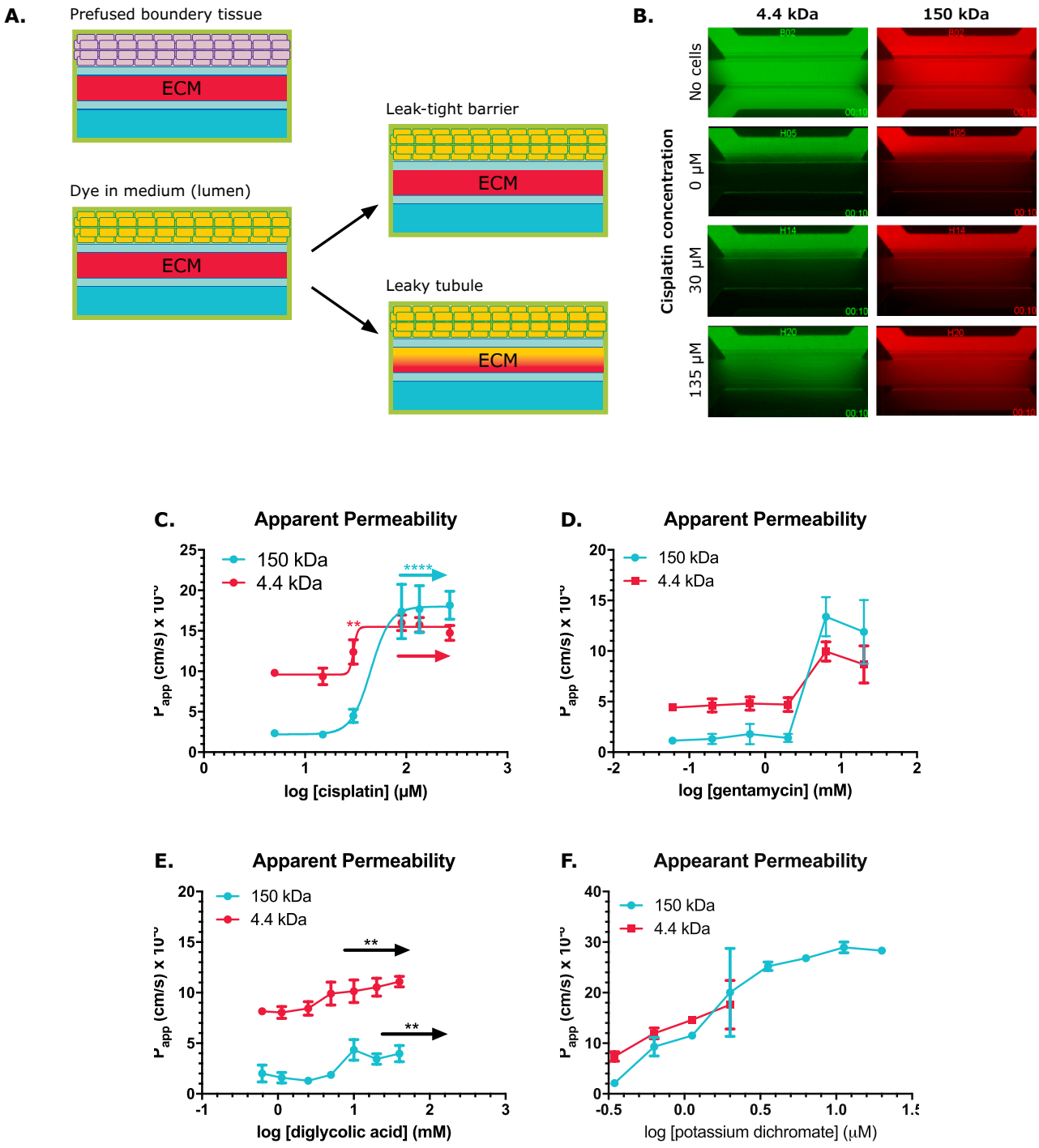

Figure 29. Increase of apparent permeability caused by exposure to nephrotoxicants. A) Schematic of the barrier integrity assay on a tubular culture in the 3-lane OrganoPlate™ platform, examined by perfusing the lumen of the tubule with dextran dyes. Leak-tight tubules retain dyes in the lumen, whereas leaky tubules allow dyes to enter the ECM channel. B) Lumen perfusion of cisplatin-treated SA7K cells was assessed with dextran dyes (4.4 kDa and 150 kDa). Barrier integrity, determined by dextran leakage into the adjacent ECM, was concentration-dependent after 48 hours of incubation with four test compounds. No difference was found at lower concentrations. Scale bar = 200 µm. C-F) A concentration-dependent loss in barrier function was monitored for all compounds.

Human Kidney Nephrotoxicity Models using Organ-on-a-Chip Devices (continued)

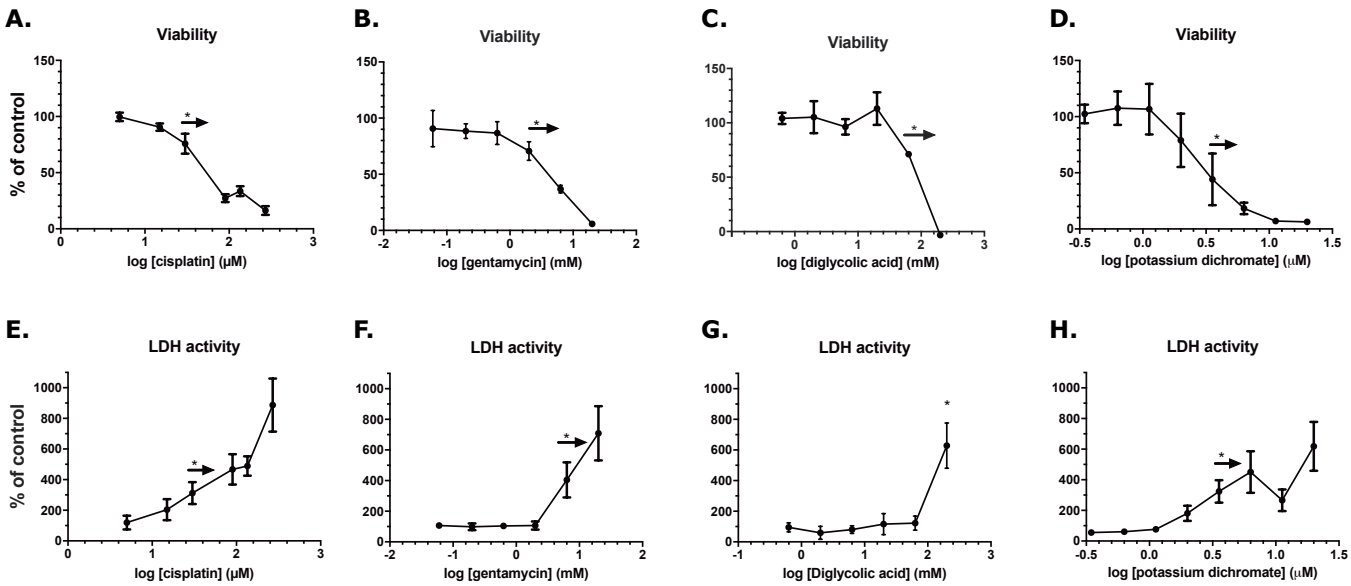

Figure 30. Viability loss and increased LDH activity after 48-hour exposure to nephrotoxicants. A-D) To quantify viability of the cells, a water-soluble tetrazolium salt-8 (WST-8) assay was used. WST-8 is reduced by the cells to an orange formazan product which can be measured directly in the OrganoPlate®with an absorbance reader at 450 nm. Viability was significantly reduced ($p<0.5$) by all four nephrotoxicants in a concentration-dependent manner compared to vehicle control. E-H) LDH activity in the medium represents the amount of cell death. The LDH assay detected a significant effect for all four compounds.

Ordering Information

Part Number	Description
MTOX1030	Kidney PTEC Control Cell (SA7K Clone)
MTOXRCSUP	RPTEC Complete Supplement sterile-filtered, suitable for cell culture
MTOXRTSUP	RPTEC Tox Supplement sterile-filtered, suitable for cell culture

Tissue Engineering Using 3D Bioprinting

3D bioprinting technology offers unprecedented versatility in developing artificially engineered, anatomically-shaped substrates with tissue-like complexity. Due to the high degree of control over structure and composition, this technology can generate precise 3D cell models and tissue constructs.

3D bioprinting has the potential to solve many critical unmet needs in medical research. Applications include cosmetics testing, drug discovery, regenerative medicine, and functional organ replacement. Creating personalized models of disease is made possible using patient-derived stem cells, such as iPSC or mesenchymal stem cells. The desired tissue construct can be supported by the appropriate application of materials, methods, and cells.

Bioinks mimic the extracellular matrix environment and contain living cells and biomaterials. These substances uniquely support cell adhesion, proliferation, and differentiation after printing through:

- Print temperatures that do not exceed physiological temperatures
- Mild cross-linking or gelation conditions
- Nontoxic bioactive compounds that promote cell viability and proliferation after printing

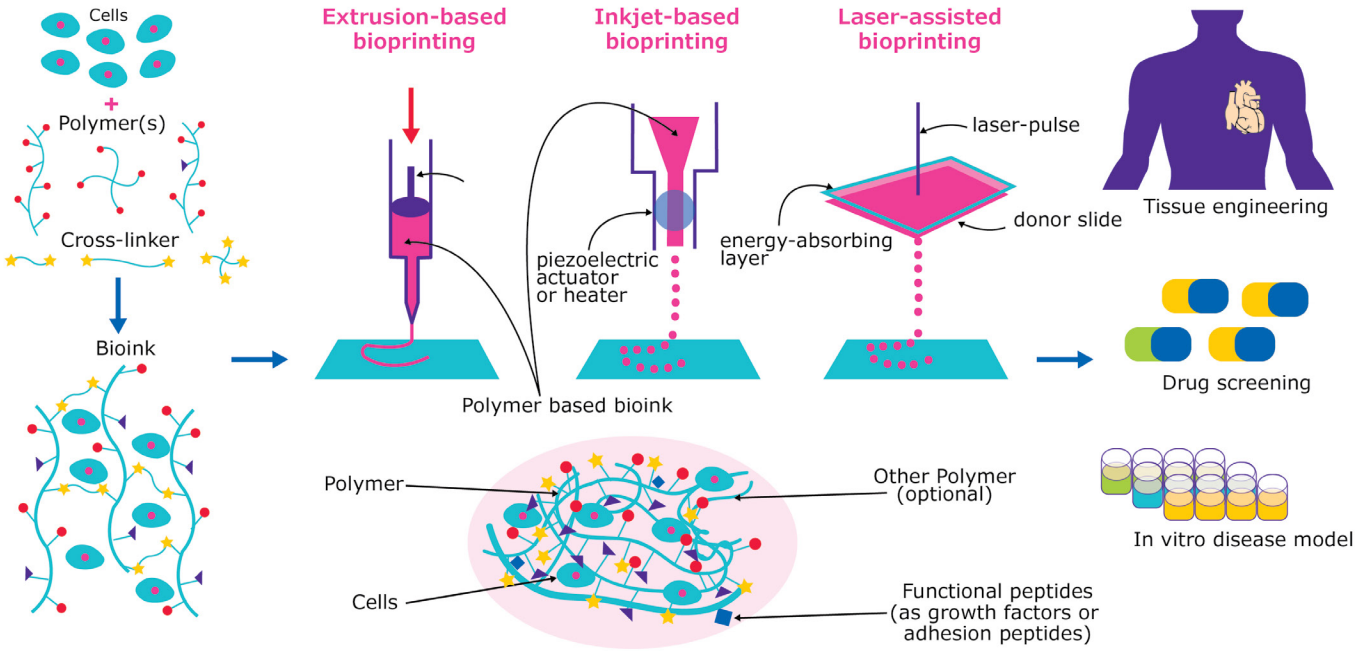

Figure 31. 3D Bioprinting of tissue and organs. Bioinks are created by combining cultured cells and various biocompatible materials. Bioinks can then be 3D bioprinted into functional tissue constructs for drug screening, disease modeling, and transplantation.

Biomaterials Commonly Used in 3D Bioprinting

Bioink Material	Overview	Advantage	Disadvantage
Agarose	Polysaccharide extracted from seaweed	Non-toxic crosslinking	Not degradable
		High stability	Poor cell adhesion
Alginate	Naturally derived biopolymer from brown algae	Mild crosslinking conditions (Ca^{2+})	Slow degradation kinetics
		Rapid gelation	Poor cell adhesion
		High biocompatibility	
Chitosan	Polysaccharide obtained from the outer skeleton of shellfish	High biocompatibility	Slow gelation rate
	Non-animal-derived chitosan can be obtained from fungal fermentation	Antibacterial properties	
Collagen	Primary structural protein found in skin and other connective tissues	High biological relevance	Acid-soluble
Decellularized ECM	Isolated extracellular matrix of a tissue from inhabiting native cells	High biological relevance	Undefined and inconsistent
		Tissue-specific	Loss of native ECM organization
		High cell survival	Low stability
Fibrin/Fibrinogen	Insoluble protein formed during blood clotting	High biological relevance	Limited printability
		Rapid gelation	
Gelatin	Protein substance derived from partial hydrolysis of collagen	High biocompatibility	Poor shape retention
		High water solubility	Limited rigidity
		Thermally reversible gelation	
Graphene	Carbon-based material; a-one-atom thick sheet of graphite	Flexible	Low biological relevance
		Electrically conductive	
Hyaluronic Acid (HA)	Non-sulfated glycosaminoglycan distributed widely throughout connective, epithelial, and neural tissues	Fast gelation	Poor stability
		Promotes cell proliferation	
Hydroxyapatite	Naturally occurring mineral form of calcium apatite found in teeth and bones	High strength and rigidity	Low printability
			Limited tissue specificity
PCL/PLA/PLGA	Biodegradable, thermoplastic polymers and/or copolymers	High strength and rigidity	Low cell adhesion and proliferation
Pluronic® F127	Poly(ethylene oxide) and poly(propylene oxide) block copolymer	Printable at room temperatures	Not suitable for long-term cell culture
		Shear thinning material	

Ordering Information

Part Number	Description
905429	TissueFab® - GelMA-UV Bioink
906891	TissueFab® - GelMA-Vis Bioink
905410	TissueFab® - GelAlg-UV Bioink
906913	TissueFab® - GelAlg-Vis Bioink
906905	TissueFab® - Sacrificial Bioink
901953	Alginate Bioink
901950	Alginate-RGD Bioink
901960	Cellulose-Alginate Bioink
901958	Cellulose-Alginate-Calcium Phosphate Bioink
901966	Cellulose-Alginate-RGD Bioink
808156	3D Printing Graphene Bioink
902403	3D Printing Hydroxyapatite Bioink
CC320	PhotoCol®-IRG, Methacrylated Collagen Hydrogel Kit
CC321	PhotoCol®-LAP, Methacrylated Collagen Hydrogel Kit
CC322	PhotoCol®-RUT, Methacrylated Collagen Hydrogel Kit
CC323	PhotoGel®-IRG, Methacrylated Gelatin Hydrogel Kit
CC324	PhotoGel®-LAP, Methacrylated Gelatin Hydrogel Kit
CC325	PhotoGel®-RUT, Methacrylated Gelatin Hydrogel Kit
CC326	PhotoHA®-IRG, Methacrylated Hyaluronic Acid Hydrogel Kit
CC327	PhotoHA®-LAP, Methacrylated Hyaluronic Acid Hydrogel Kit
CC328	PhotoHA®-RUT, Methacrylated Hyaluronic Acid Hydrogel Kit

Isolation and Culture of Malignant Primary Cancer Cells from PDX and Human Tumor Samples

The unique cellular features of malignant primary cancer cells can present challenges for cell isolation and culture. Traditional tumor cell media lack specificity to support malignant cells, including patient tumor samples or patient-derived xenografts (PDXs) and are not cost-effective.

The PromoCell™ Primary Cancer Culture System consists of Primary Cancer Cell Medium D-ACF and the NCCD-Reagent, and was designed to be the first universally-applicable and cost-effective solution for *in vitro* isolation of long-term primary tumor culture from patient tumor samples or PDXs. The advanced PromoCell™ Primary Cancer Culture System selection process uses a proprietary NCCD-Reagent treatment and an optimized animal-free cancer cell culture media.

A. **B.**

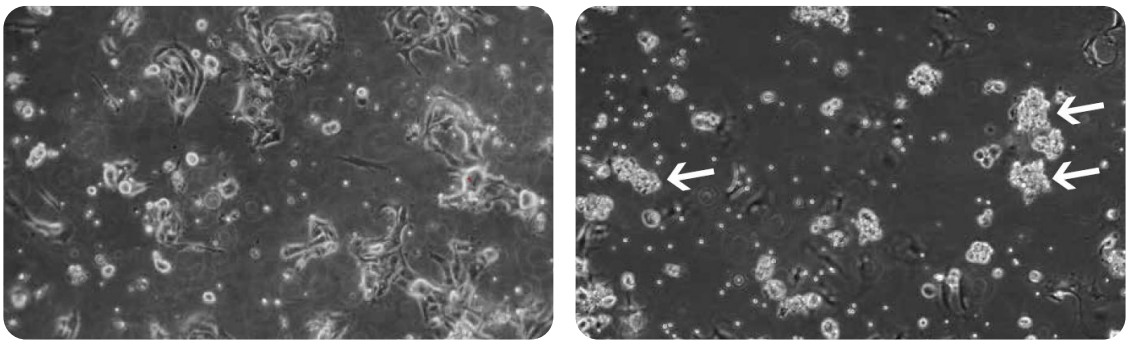

Figure 32. Early-stage primary culture of lung squamous carcinoma cells in the Primary Cancer Cell Medium D-ACF. Residual erythrocytes, fibroblast- and epithelial- like adherent cells, and floating single suspension cells are observed on day two (left). Prominent formation of floating multicellular cell aggregates(white arrows) is observed on day 11 (right). The culture was used for primary aggregate separation on day 13, and also cultured in a new flask parallel to the original sample.

A. **B.**

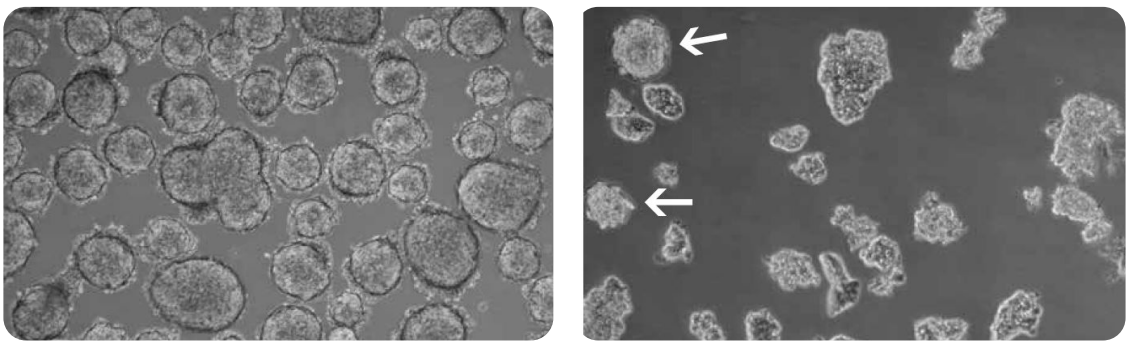

Figure 33. Primary culture derived from a low-grade small cell lung cancer and cultured using the PromoCell™ Primary Cancer Culture System. A) The primary isolate was obtained after four weeks as a floating sphere-forming culture, which persisted in a near-quiescent state even after six months. B) Adding extra growth factors elicited significant expansion in the latent sphere culture with a doubling time of 3-4 weeks. Note that some spheres persisted under these modified culture conditions (white arrows), while the larger part of the culture proliferated as floating planar multicellular 2D sheets, which is a prototypical growth pattern for SCLC cells *in vitro*.

Ordering Information

Part Number	Description
C-28081	Primary Cancer Culture System 250 ml
C-43080	NCCD Reagent 2 ml

Analyzing the Effect of Matrix Stiffness on Regulating Cellular Behavior Using CytoSoft® Elastic Modulus Plates

Using a multi-step mechanotransduction process, cells can sense and respond to mechanical stimuli by converting biochemical signals to specific cellular responses. In an organism, however, changes in matrix viscosity can influence the extracellular matrix properties in which cells reside. In order to create more relevant *in vitro* cell models, researchers have begun to grow cells on softer 3D hydrogels and substrates that more closely represent the stiffness of native tissues.

Tissues and organs have much lower (0.2-64 kPa) elastic modulus (measurable stiffness) when compared to tissue culture plasticware ($1x10^7$ kPa), which may influence cell differentiation potential. For example, stiffer substrates can alter the differentiation potential of human mesenchymal stem cells to favor bone formation over cartilage and adipose tissues.

CytoSoft® elastic modulus plates are used to culture cells on substrates, covering a broad physiological range of stiffness (0.2 kPA - 64 kPa). A thin layer of specially formulated biocompatible silicone is on the bottom of each well. Precise and consistent elastic modulus supports the formation of covalent bonds with amines on proteins on the surfaces of the gel.

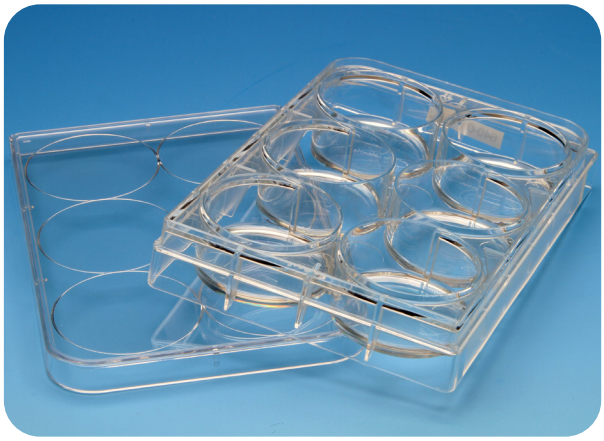

Figure 34. CytoSoft® plates

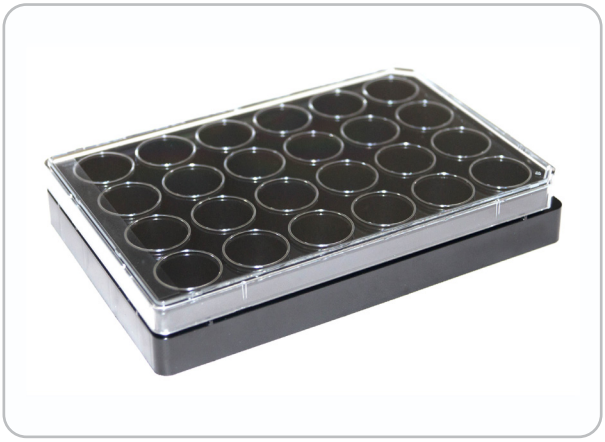

Figure 35. CytoSoft® imaging plates

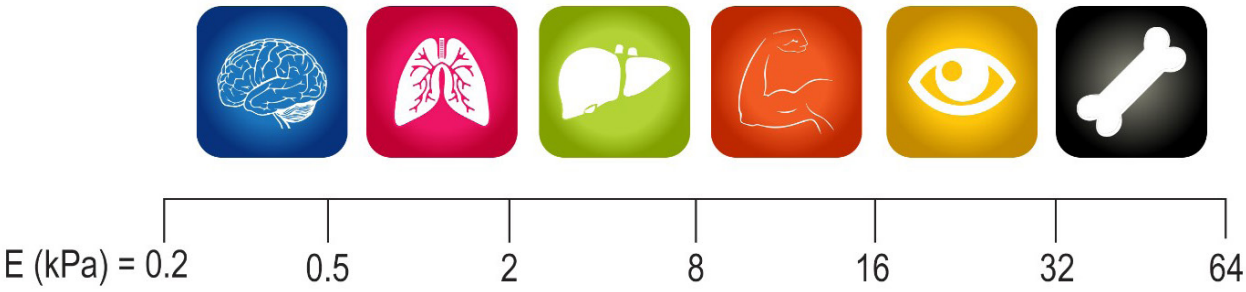

Figure 36. Native matrix stiffnesses of various *in vivo* tissues.

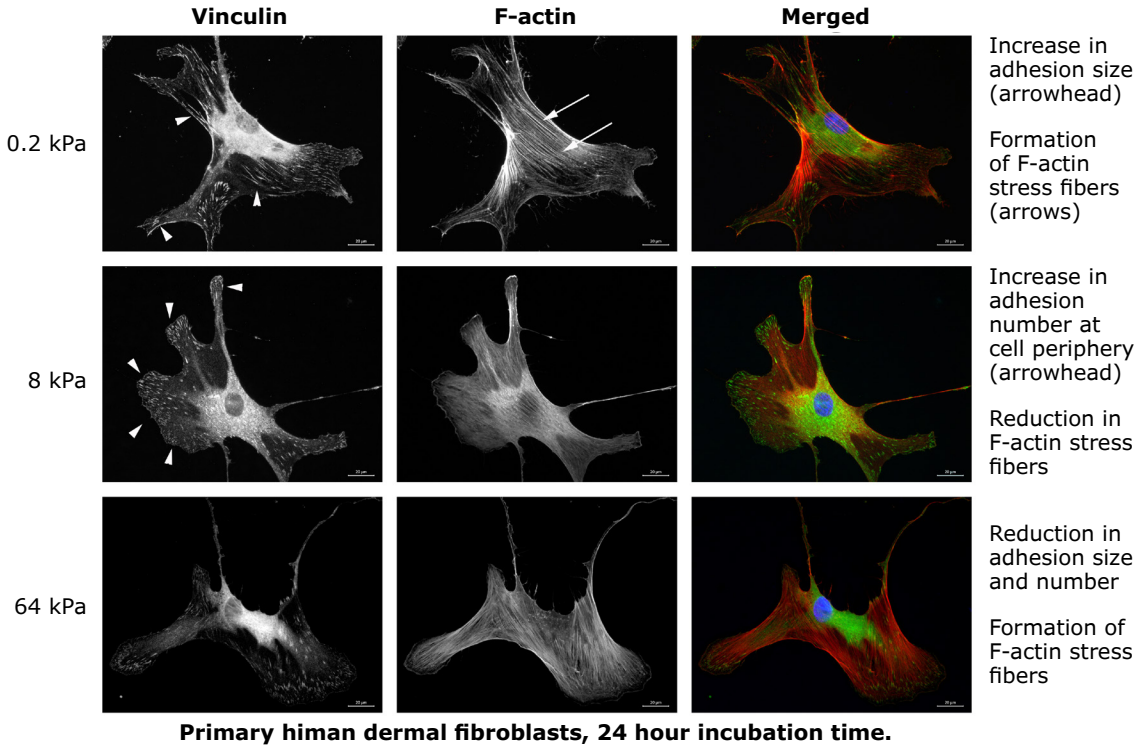

Figure 37. Primary human dermal fibroblast matrix stiffness optimization. Optimal matrix stiffness for dermal fibroblasts (8 kPa) showed a reduction in F-actin stress fibers and increased cell adhesions (as demonstrated by vinculin) when compared with an elastic modulus of 0.2 or 64 kPa.

Ordering Information

Part Number	Description
5165	CytoSoft® Elastic Moduli 0.2 kPa
5140	CytoSoft® Elastic Moduli 0.5 kPa
5141	CytoSoft® Elastic Moduli 2 kPa
5142	CytoSoft® Elastic Moduli 8 kPa
5143	CytoSoft® Elastic Moduli 16 kPa
5144	CytoSoft® Elastic Moduli 32 kPa
5145	CytoSoft® Elastic Moduli 64 kPa
5190	CytoSoft® Discovery Kit Multiple Elastic Moduli, (0.2, 0.5, 2, 8, 16, 32, 64 kPa)
CC306	CytoSoft® Imaging 24-Well Plate (Elastic Moduli 0.2 kPa)
CC307	CytoSoft® Imaging 24-Well Plate (Elastic Moduli 0.5 kPa)
CC308	CytoSoft® Imaging 24-Well Plate (Elastic Moduli 2 kPa)
CC309	CytoSoft® Imaging 24-Well Plate (Elastic Moduli 8 kPa)
CC310	CytoSoft® Imaging 24-Well Plate (Elastic Moduli 16 kPa)
CC311	CytoSoft® Imaging 24-Well Plate (Elastic Moduli 32 kPa)
CC312	CytoSoft® Imaging 24-Well Plate (Elastic Moduli 64 kPa)
CC313	CytoSoft® Imaging 96-Well Plate (Elastic Moduli 0.2 kPa)
CC314	CytoSoft® Imaging 96-Well Plate (Elastic Moduli 0.5 kPa)
CC315	CytoSoft® Imaging 96-Well Plate (Elastic Moduli 2 kPa)
CC316	CytoSoft® Imaging 96-Well Plate (Elastic Moduli 8 kPa)
CC317	CytoSoft® Imaging 96-Well Plate (Elastic Moduli 16 kPa)
CC318	CytoSoft® Imaging 96-Well Plate (Elastic Moduli 32 kPa)
CC319	CytoSoft® Imaging 96-Well Plate (Elastic Moduli 64 kPa)

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- The CellASIC® live cell imaging system and live cell dyes
- The Scepter™ instrument for rapid, accurate Coulter-based cell counting

Cell transfection

- Reagents for gentle transfection of sensitive primary cells

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- Dyes for antibody, protein, and nucleic acid labeling
- Fluorescent cell stains and indicators

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- Consistent, highly validated ZooMAb® recombinant antibodies
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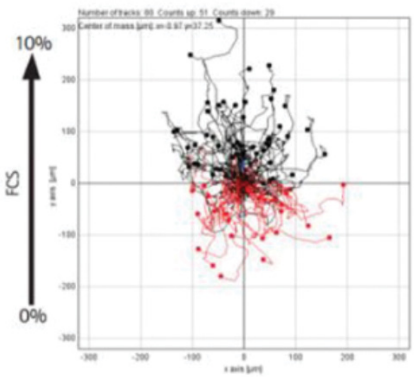
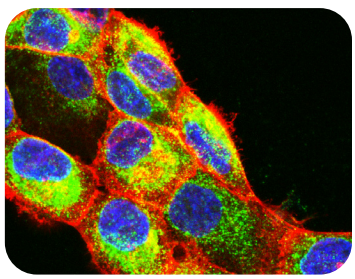


Migration of HT-1080 cells toward an increasing FBS gradient



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