

CORNING

EuroClone[®]
serving science through innovation

Rock the Science of 3D!

Corning Life Science





3D Cell Culture mimics the natural environment of cells

2D
CELL CULTURE

plays a pivotal role in drug discovery and cell biology research, but it is limited in the context of *in vivo* conditions.

In vivo-like 3D models more effectively support biologically relevant experiments.

3D
CELL CULTURE



2D or 3D? It's no longer a question

Why have so many research scientists embraced 3D cell culture? Because cells grown in 3D more closely mimic *in vivo* behavior in tissues and organs than cells grown in a 2D culture model. 3D cell culture environments create more biologically relevant models for drug discovery which may lead to more predictive results, higher success rates for drug compound testing, a faster path to market, and reduced development costs.

Attribute	2D	3D
<i>Growth Substrate</i>	Rigid, inert	Mimics natural tissue environment
<i>Cell Shape Growth</i>	Loss of cell polarity and altered shape	Maintains <i>in vivo</i> -like morphology and polarity
<i>Architecture</i>	Not physiological, cells partially interact	Physiological, promotes close interaction between cells, ECMS, and growth factors
<i>Growth Factor Diffusion</i>	Rapid	Slow – biochemical gradients regulate cell-to-cell communication and signaling
<i>Gene Expression</i>	Different patterns of gene expression	Maintenance of <i>in vivo</i> -like expression patterns

Tools for Efficient 3-Dimensional Cell Culture

Corning provides proven tools for the critical steps of 3D cell culture.



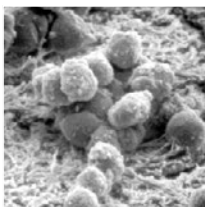
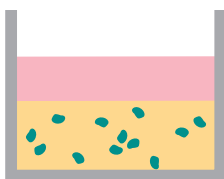
Proliferating cells



Primary cells



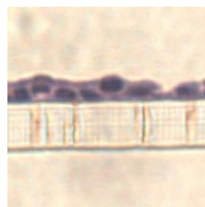
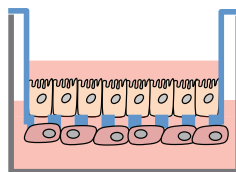
Cell Differentiation in a Matrix



Applications

- ▶ Cell function
- ▶ Cell-ECM interaction
- ▶ Compound screening with organoids

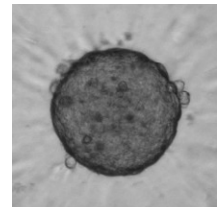
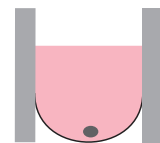
Cell Differentiation on Porous Membranes



Applications

- ▶ Cell function
- ▶ Cell motility
- ▶ Co-culture models
- ▶ Compound screening based on cell barrier

Cell Aggregation as Spheroids



Applications

- ▶ Cell function
- ▶ Cell-cell interaction
- ▶ Co-culture models
- ▶ Compound screening with spheroids



Break the 3D barrier
Get there fast with Corning

Select Products for 3-Dimensional Cell Culture

Ultra-Low Attachment (ULA) Surface Cultureware

Cat. No.	Description	Qty/Pk	Qty/Cs
CC4515	Corning® Spheroid microplate, 96-well, black/clear round bottom, with lid, ULA surface, sterile, individually wrapped	1	5
CC4516	Corning Spheroid microplate, 384-well, black/clear round bottom, with lid, ULA surface, sterile, individually wrapped	1	5
CC3471	Costar® 6-well clear plate, ULA surface, with lid, individually wrapped	1	24
CC3474	Corning 96-well clear microplate, flat bottom, ULA surface, with lid, individually wrapped	1	24
CC7007	Corning 96-well clear microplate, round bottom, ULA surface, with lid, individually wrapped	1	24
CC3814	Corning 75 cm ² U-shaped flask, canted neck, ULA surface, vented cap	4	24

Permeable Supports

CC3412	Transwell® Inserts in 6-well plates, 24 mm, 0.4 µm, Tissue Culture (TC)-treated, PC membrane, sterile	6	24
CC3479	Netwell™ Inserts in 6-well plates, 24 mm, 74 µm, PTFE, sterile	6	48
CC3384	HTS Transwell 96-well with receiver plate and lid, 8.0 µm, TC-treated (membrane), PET membrane, bulk	4	8

Cell Strainer

CC352340	CORNING 40 µm Cell strainer	1	50
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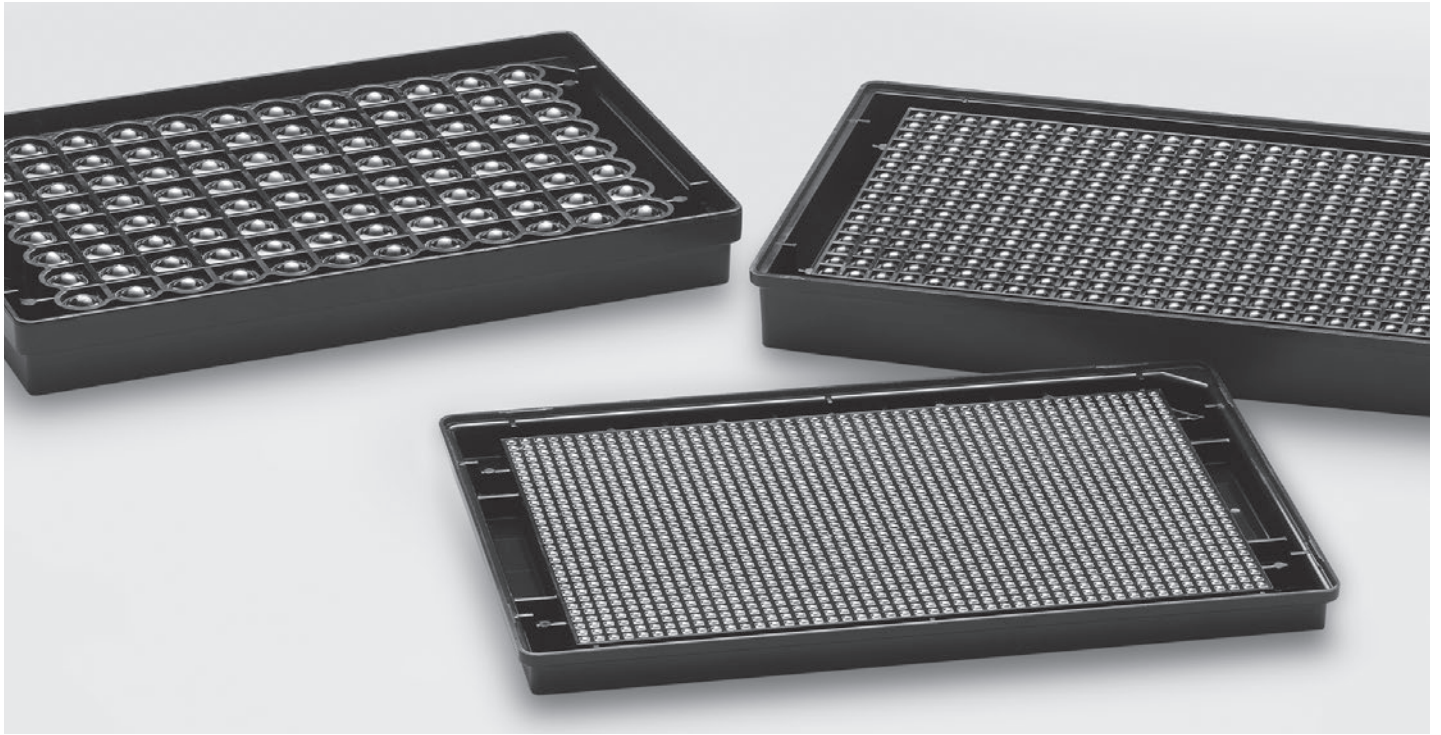
Corning Cooling Systems and Accessories

CC432003	Corning CoolCell® LX, orange freezing container, for 12 x 1 mL or 2 mL cryogenic vials	1	1
CC432008	Corning CoolCell FTS30, green freezing container, for 30 x 1 mL or 2 mL cryogenic vials	1	1
CC432014	Corning CoolBox™ XT Starter complete, low profile, open platform ice-free cooler	1	1
CC432021	Corning CoolBox XT, purple cooling and freezing workstation, single capacity	1	1
CC432034	Corning CoolRack® M6, gray holds 6 x 1.5 or 2 mL microcentrifuge tubes	1	1
CC432044	Corning CoolRack M90, gray, holds 90 x 1.5 or 2 mL microcentrifuge tubes	1	1
CC432070	Corning CoolSink® XT96F, flat bottom plate module	1	1
CC432071	Corning CoolSink XT 96U, 96-well U-bottom microplate module	1	1

Equipment

CC6749	Corning Cell Counter	1	1
CC4099	Corning Stripettor™ Ultra Pipet Controller	1	1
CC6791	Corning LSE™ Benchtop shaking incubator with platform, 230V (EU Plug)	1	1





Citations on Corning® Spheroid Microplates with Ultra-Low Attachment Surface

Spheroids are organized and functional 3-Dimensional cell aggregates that better mimic the structure of cells in tissues compared to 2D cell culture. This characteristic makes spheroids a powerful tool to study more physiologically relevant cell behavior, resulting in more biologically relevant results.

2D versus 3D

The *in vivo*-like 3D structure of spheroids, in comparison with 2D cell culture, closely resembles the natural environment of cells.

1. Mathews Griner LA, et al. Large-scale Pharmacological Profiling of 3D Tumor Models of Cancer Cells

Cell Death & Disease 7/12:e2492, 2016.

In this study, the compound-induced pharmacologic responses of cells cultured in either in 2D or 3D were analyzed and the authors show that the responses are crucially different between the culture methods, notably in the magnitude of their synergistic cytotoxic effects.

2. Stock K, et al. Capturing Tumor Complexity *In Vitro*: Comparative Analysis of 2D and 3D Tumor Models for Drug Discovery

Scientific Reports, 6:28951, 2016.

Choosing a proper model to conduct drug discovery can be challenging. The authors present a guide to select the most suitable *in vitro* model for specific assay types. Includes a review of three different cell carcinoma cell lines and methods for 3D cell culture.

3. Senkowski W, et al. Large-Scale Gene Expression Profiling Platform for Identification of Context-dependent Drug Responses in Multicellular Tumor Spheroids

Cell Chemical Biology, 23:1428-1438, 2016.

The combination of high throughput gene expression profiling with tumor spheroid-based drug-screening is a potent tool for identifying promising drug candidates. The authors highlight the necessity to implement gene expression profiling as a measure for cellular response profiles in order to evaluate and understand the effect of compounds. Using this approach, the authors identified a synergistic effect of two compounds on quiescent spheroids not detected in 2D monolayers. This illustrates the importance of using 3D cell culture and spheroids over 2D cultures.

4. de Hoogt R, et al. Protocols and Characterization Data for 2D, 3D, and Slice-based Tumor Models from the PREDECT Project

Sci Data 4:170170, 2017.

The PREDECT consortium presents a characterization of *in vitro* models of three different solid tumor types (2D, 3D and tumor slice models). Detailed protocols and procedures were developed for the three tumors and are presented in this work.

Drug Discovery

Spheroids enable to determine the efficacy and toxicity of a drug on human cells in a more *in vivo*-like context compared to traditional 2D cell cultures. This alignment of cellular models with pre-clinical animal models and patient tumors can accelerate the screening and evaluation of new drug candidate.

1. Baek N, et al. Monitoring the Effects of Doxorubicin on 3D-Spheroid Tumour Cells in Real-Time

Onco Targets Ther. 9:7207-7218, 2016.

In this work, 12 tumor cells lines were tested in a 3D-spheroid real-time culture assay model for the effects of doxorubicin, a known cytostatic drug. It was demonstrated that the degree of sensitivity against this drug in traditional 2D cultures was not observed in 3D cell cultures: IC₅₀ values usually were higher compared to conventional culture. As drugs often exhibit variation in their effects on patients or animals compared to the 2D tissue culture results, the 3D models may help to better understand sensitivities and tolerances.

2. Fryknas M, et al. Iron Chelators Target Both Proliferating and Quiescent Cancer Cells

Scientific Reports, 6:38343, 2016.

The most common cancer drugs target the cell-cycle pathway. Quiescent cells, due to their nature, are thus more resistant to cancer drugs. In this study the authors have identified VLX600, an iron chelator, as potential compound for chemotherapy. The efficacy of the compound was evaluated by the survival rate of quiescent cells in a spheroid.

3. Sirenko O, et al. Phenotypic Characterization of Toxic Compound Effects on Liver Spheroids Derived from iPSC Using Confocal Imaging and Three-Dimensional Image Analysis

Assay and Drug Development Technologies, 14:381-394, 2016.

The authors developed a phenotypic assay read out for analyzing 3D model systems formed with human iPSC-derived hepatocytes. The effect of compounds was evaluated with the use of a confocal microscope scoring multi-parametric cellular information. This set up is suitable for high throughput screening to evaluate hepatotoxicity *in vitro*.

4. Martinez NJ, et al. A High-Throughput Screen Identifies 2,9-Diazaspiro[5.5]Undecanes as Inducers of the Endoplasmic Reticulum Stress Response with Cytotoxic Activity in 3D Glioma Cell Models

PloS One, 11, e0166506, 2016.

Cancer cells were shown to be more susceptible to stress of the endoplasmic reticulum, which results in apoptosis. The efficacy of small molecules to induce stress of the endoplasmic reticulum, which was, amongst others, analyzed by the survival rate of treated cells in spheroids.

5. Hagemann J, et al. Spheroid-based 3D Cell Cultures Enable Personalized Therapy Testing and Drug Discovery in Head and Neck Cancer

Anticancer Research 37:2201-2210, 2017.

Standardized chemotherapy shows varying response rates with unpredictable efficacy, due to tumor heterogeneity and the different, individual mutations. Personalized tumor therapy can increase the efficacy of the treatment, by addressing the specific characteristics of the patient's tumors. Therefore, the authors developed a model using spheroids subjected to standardized, high throughput *in vitro* assays, in which spheroids were grown and analyzed for therapy susceptibility. The ultimate goal is to grow spheroids from patient cells and analyze the individual therapy susceptibility.

Fundamental Research

The microenvironment in spheroids mimics the *in vivo* physiology. Therefore, spheroids represent a valuable tool for research on the development and homeostasis of organs and tissues and provide crucial insights into the formation and structural composition of tumors.

1. Sirenko O, et al. High-Content Assays for Characterizing the Viability and Morphology of 3D Cancer Spheroid Cultures

Assay Drug Dev Technol 13/7:402-414, 2015.

Researchers use 96- or 384-well Ultra-Low Attachment surface-coated spheroid microplates for the generation of 3D spheroids from three different human tumor cell lines. They were able to demonstrate that these spheroids could be analyzed with high content imaging using low and high magnifications. The relevance of the model was confirmed by testing 119 known approved anticancer drugs on one of the cell lines.

2. Comley J. Spheroids Rapidly Becoming a Preferred 3D Culture Format

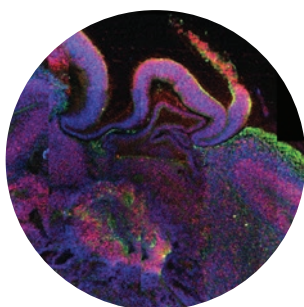
Drug Discovery World Spring 2017, 31-49, 2017.

In this article, several current approaches for 3D spheroid cultures, including Corning spheroid microplates, are reviewed and discussed.

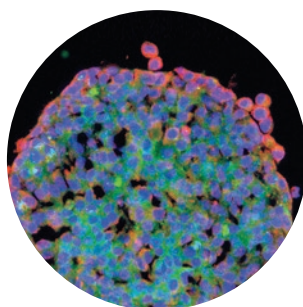
3. Molla A, et al. Unsuccessful Mitosis in Multicellular Tumor Spheroids

Oncotarget 25/8:28769-28784, 2017.

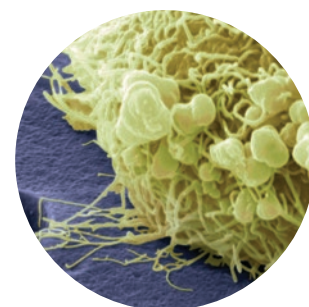
Binucleated cells, originating from unsuccessful mitosis, are frequently observed in human tumors. The authors of this article demonstrate that certain human tumor cells, cultured as spheroids in 3D, also exhibit unsuccessful mitosis. These multicellular tumor spheroids however, still remain sensitive to certain mitotic drugs. In conclusion, multicellular tumor spheroids can be used as a relevant model for drug testing.



Cerebral organoid culture using Corning Matrigel matrix.



Lung tumor cells and primary lung fibroblasts grown in a Corning spheroid microplate.



Migration/invasion assays as performed on Corning permeable supports.

4. Senkowski W. High-throughput Screening Using Multicellular Tumor Spheroids to Reveal and Exploit Tumor-specific Vulnerabilities

Doctoral Thesis, Uppsala: Acta Universitatis Upsaliensis, p.50, 2017.

Senkowski describes in his online-published Ph.D. thesis the use of multicellular tumor spheroids, containing both proliferating and quiescent cells, for more consistent drug testing and screening, when compared to conventional two-dimensional tissue culture models. In particular, it is demonstrated in this work that previously unknown tumor-specific vulnerabilities can be detected using the more *in vivo*-like 3D model.

5. Kota S, et al. A Novel Three-Dimensional High-throughput Screening Approach Identifies Inducers of a Mutant KRAS Selective Lethal Phenotype

Oncogene, 2018.

The authors demonstrate the necessity of evaluating potential drugs in the 3D cell environment. Spheroids were generated of isogenic cell lines, in which genes of the RAS proteins were mutated. RAS proteins play an important role in the extracellular-intracellular signaling pathways and are found to be commonly mutated in cancer. Spheroids were subjected to Proscillaridin A, which was identified as selective inhibitor of cells in which the RAS protein KRAS was mutated. The discovery of Proscillaridin A would have been missed out if the efficacy would have been tested in standard 2D culturing methods.

6. Madoux F, et al. A 1536-well 3D Viability Assay to Assess the Cytotoxic Effect of Drugs on Spheroids

SLAS Discovery: Advancing Life Sciences R & D, 22:516-524, 2017.

In this work the authors from the SCRIPPS Institute investigate the cytotoxic effects of about 3.300 drug compounds in a 3D assay using spheroids which were generated in a 1536-well microplate custom designed by Corning. The plate features an Ultra-Low Attachment surface which allowed for the parallel formation, measurement of size as well as viability testing of the spheroids in a robust and reproducible manner.

Featured Corning Products

- ▶ 384-well black/clear round bottom spheroid microplate with Ultra-Low Attachment surface (Corning Cat. Nos. CC3830, CC4520, and CC4516)
- ▶ 96-well spheroid microplates with Ultra-Low Attachment surface (Corning Cat. Nos. CC4515 and CC4520)

For more specific information on claims, visit the Certificates page at www.corning.com/lifesciences.

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