

CORNING

Rock the Science of 3D Tutorial

3D Technologies for Advanced Cancer Models

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Welcome and Introduction



3D Technologies for Advanced Cancer Models

Presented by: Audrey Bergeron, Applications Specialist, Corning Life Sciences

Corning leads the market in laboratory consumables through a family of respected brands

A portfolio trusted and endorsed by labs and scientists worldwide.

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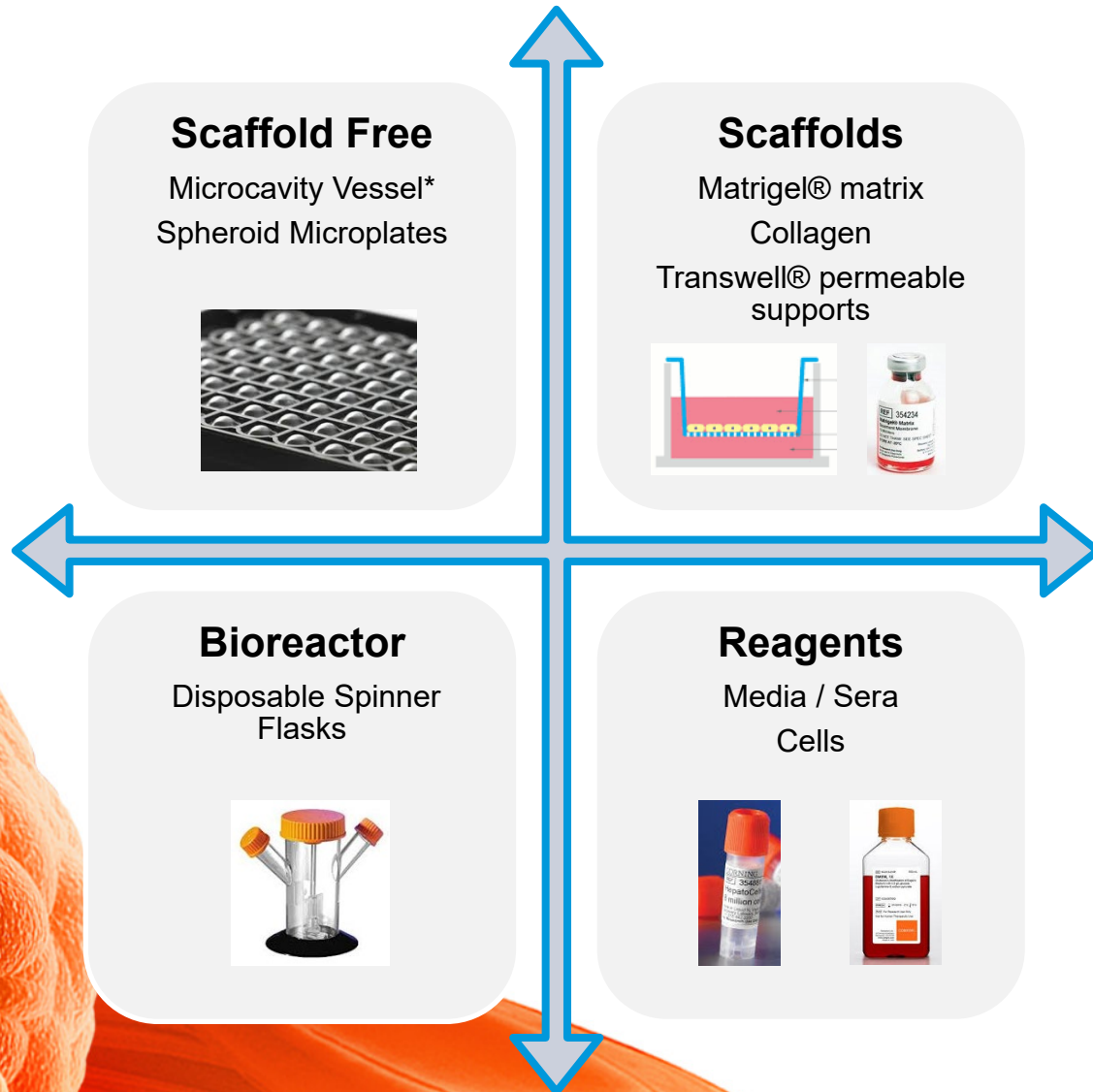
AXYGEN®

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3D Cell Culture: From Promise to Reality



3D cell culture utilizes a broad range of Corning products



Corning is a leader in:

- Spheroid Microplates
- Permeable Supports
- Matrigel matrix
- Spinner Flasks
- Serum



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*SLAS Technology: Translating Life
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Advanced Models for 3D Cancer Applications

Audrey Bergeron
Applications Scientist II
Corning Life Sciences



Overview

Introduction to 3D Cell Culture

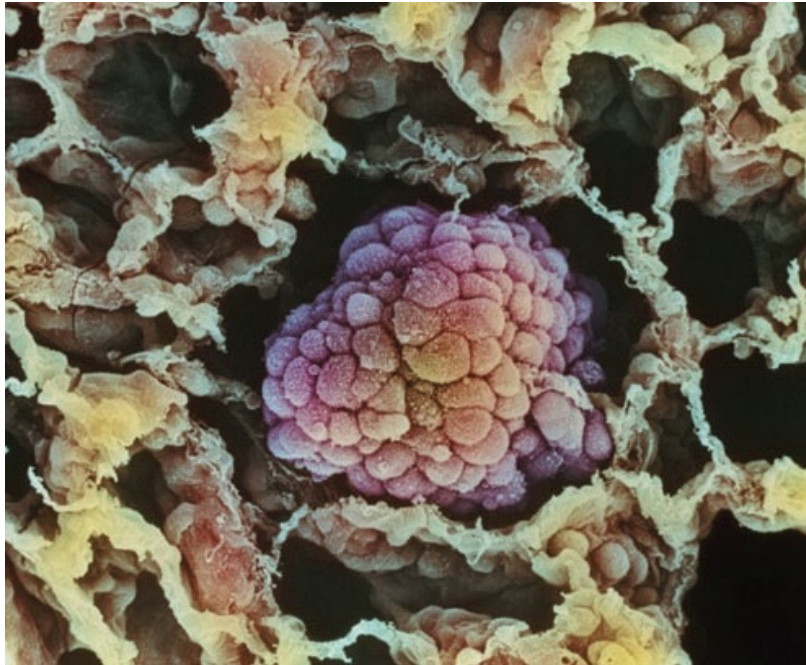
- Why 3D?
- Methods for 3D Culture
- Assay Techniques in Spheroid Microplates

Modeling Cancer in an Assay Compatible Format

- Spheroid Screening
- Co-culture
- Immune Cell Therapy

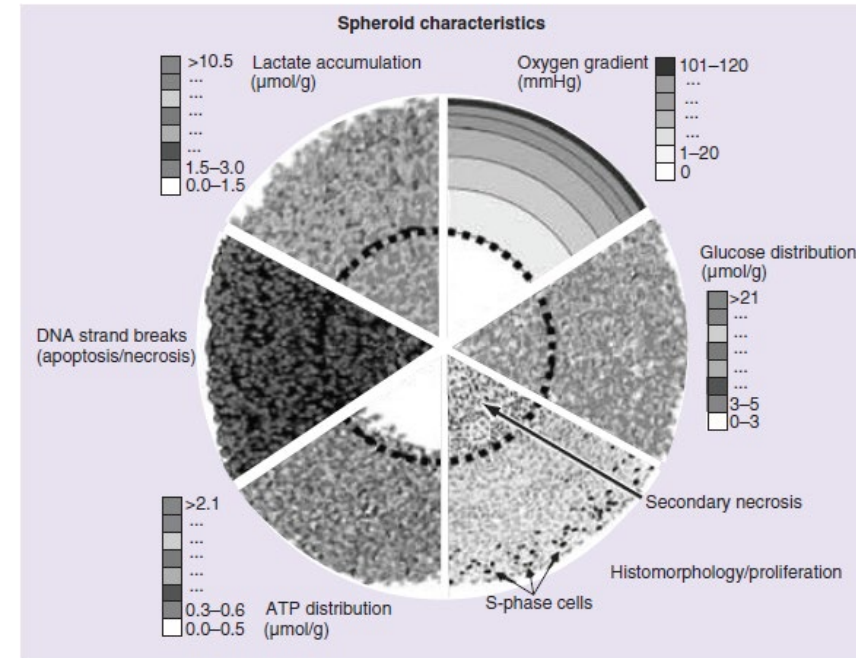
Why 3D?

3D is more physiologically relevant than 2D



A scanning electron micrograph image showing the 3D nature of a small cancerous tumor within a human lung.

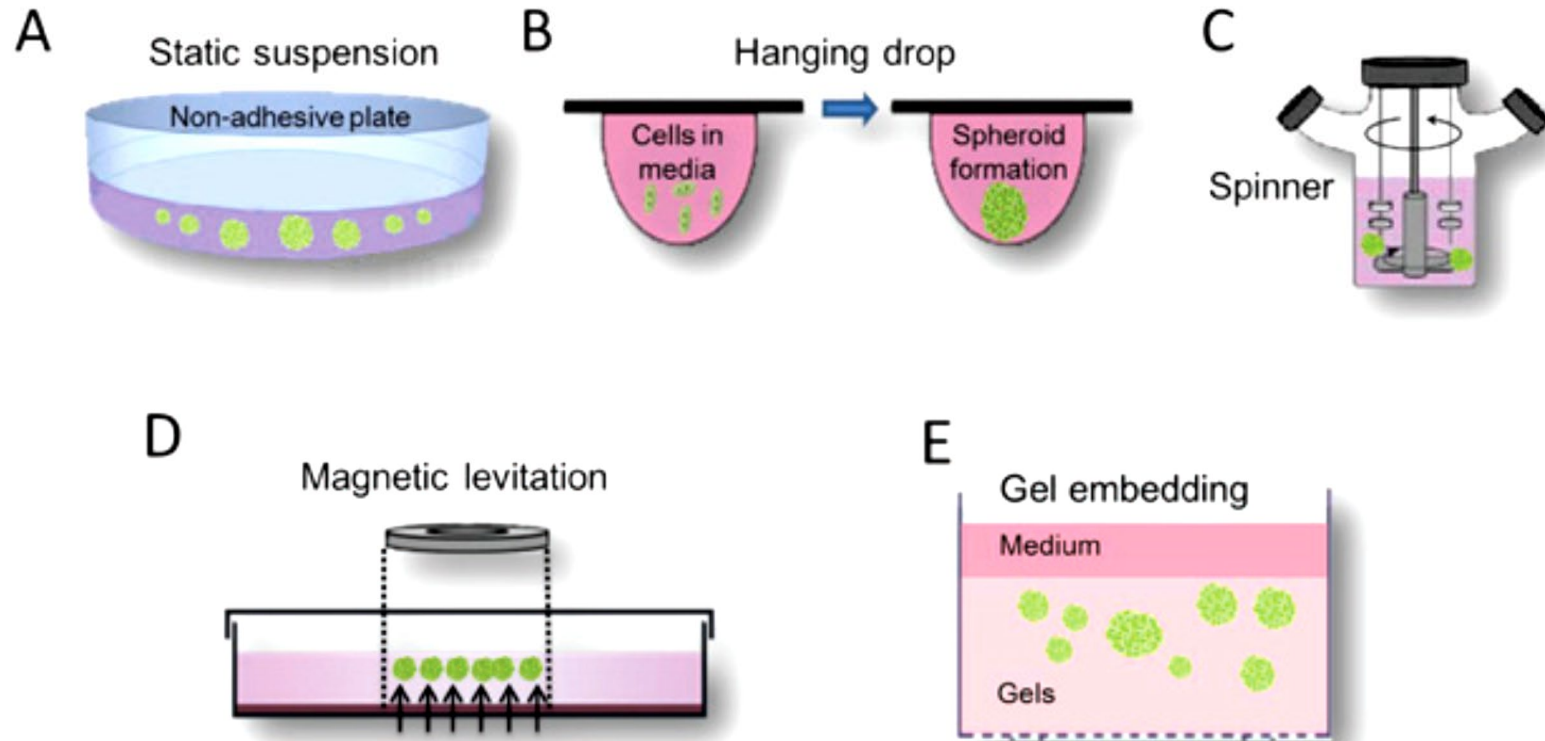
V.S. Nirmalanandhan, et al., Activity of Anticancer Agents in a Three-Dimensional Cell Culture Model. ASSAY and Drug Development Technologies. 8(5):581-590 (2010).



Various gradients within a multicellular tumor spheroid.

Benien & Swami. 3D Tumor Models: History, Advances & Future Perspectives. Future Oncol. (2014) 10(7):1311-1327.

Various Methods Used for 3D Formation and Culture

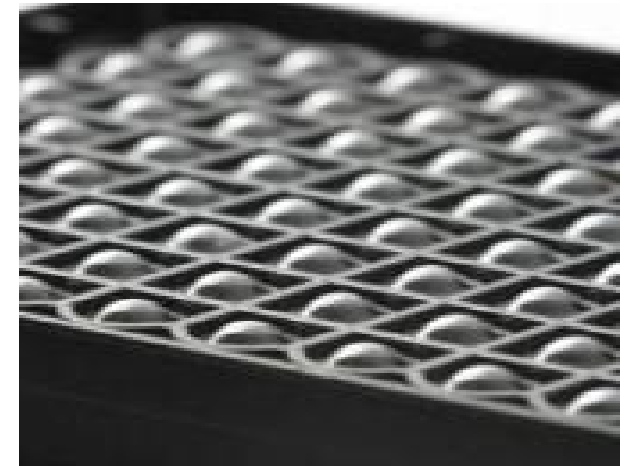
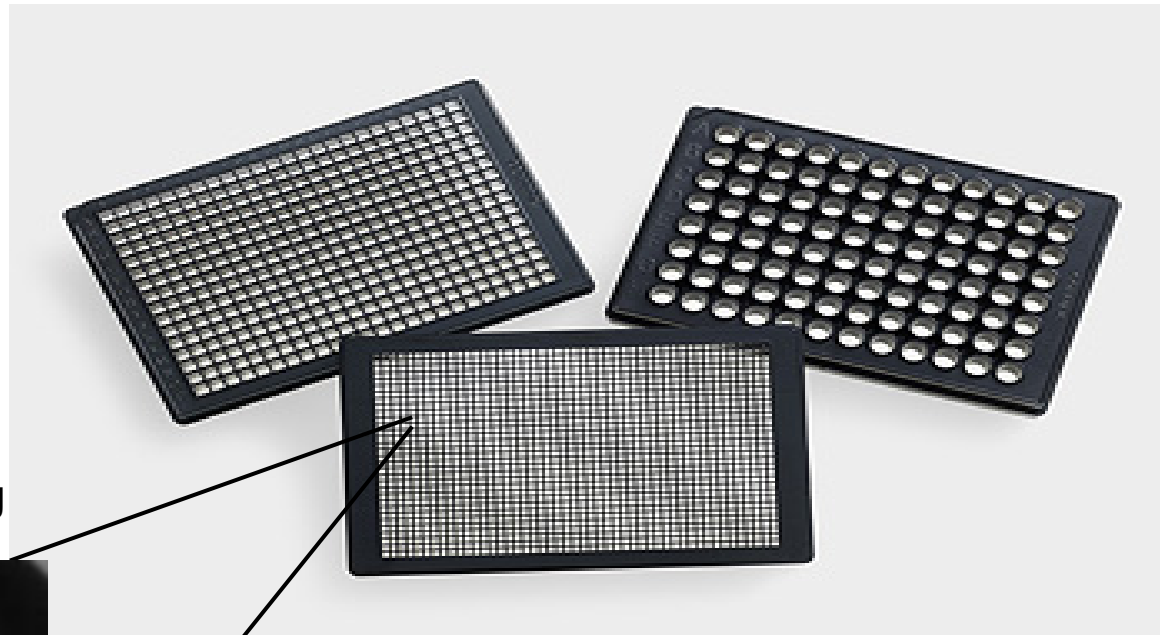


Adapted from Lv, D., Hu, Z., Lu, L., Lu, H., Xu, X. "Three-dimensional cell culture: A powerful tool in tumor research and drug discovery (Review)". *Oncology Letters* 14.6 (2017): 6999-7010.

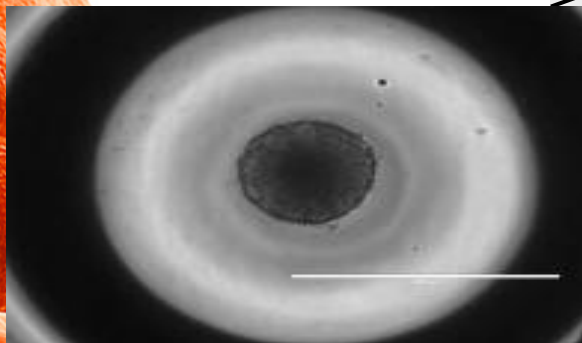
Corning® Spheroid Microplates

Corning Ultra-Low Attachment surface and unique round well-bottom design enable the formation and growth of a single, uniform spheroid per well with reproducible size.

Standard ANSI/SBS footprint dimensions for 96-well, 384-well and 1536-well formats



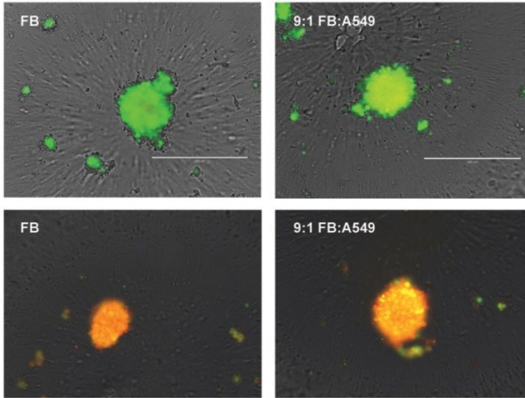
Clear bottom for visualization and imaging



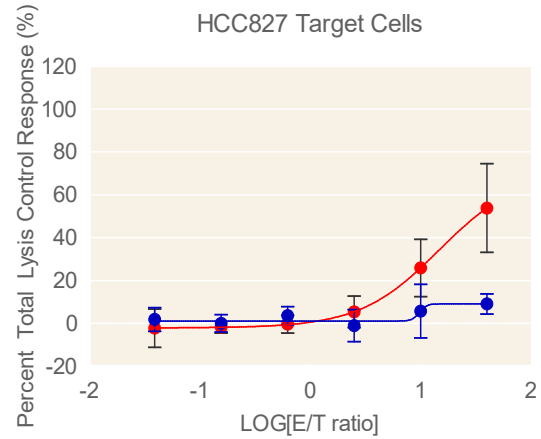
Black sidewalls to reduce cross-talk and background noise in fluorescent- and luminescent-based assays

Spheroid Plate Detection Methods

Fluorescence

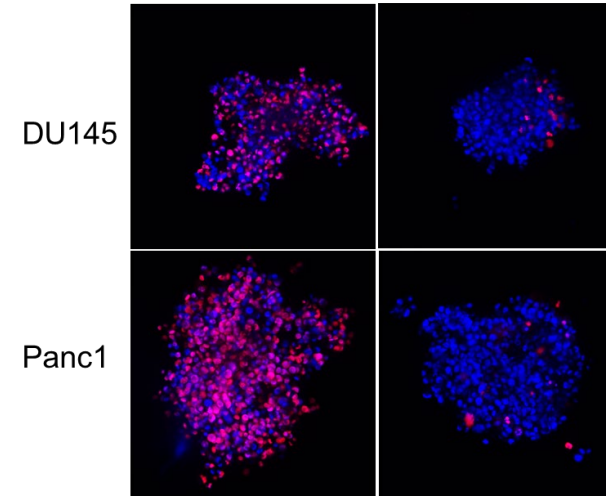


Luminescence

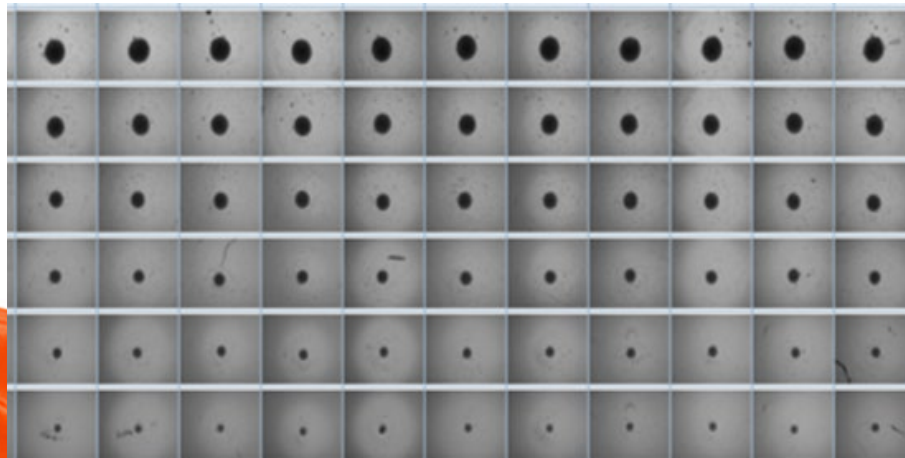


High Content

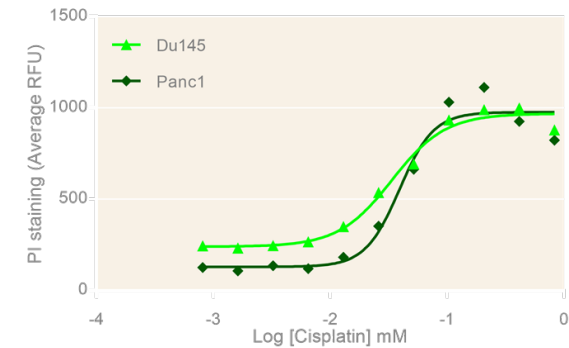
83 mM 0 mM



Bright-field

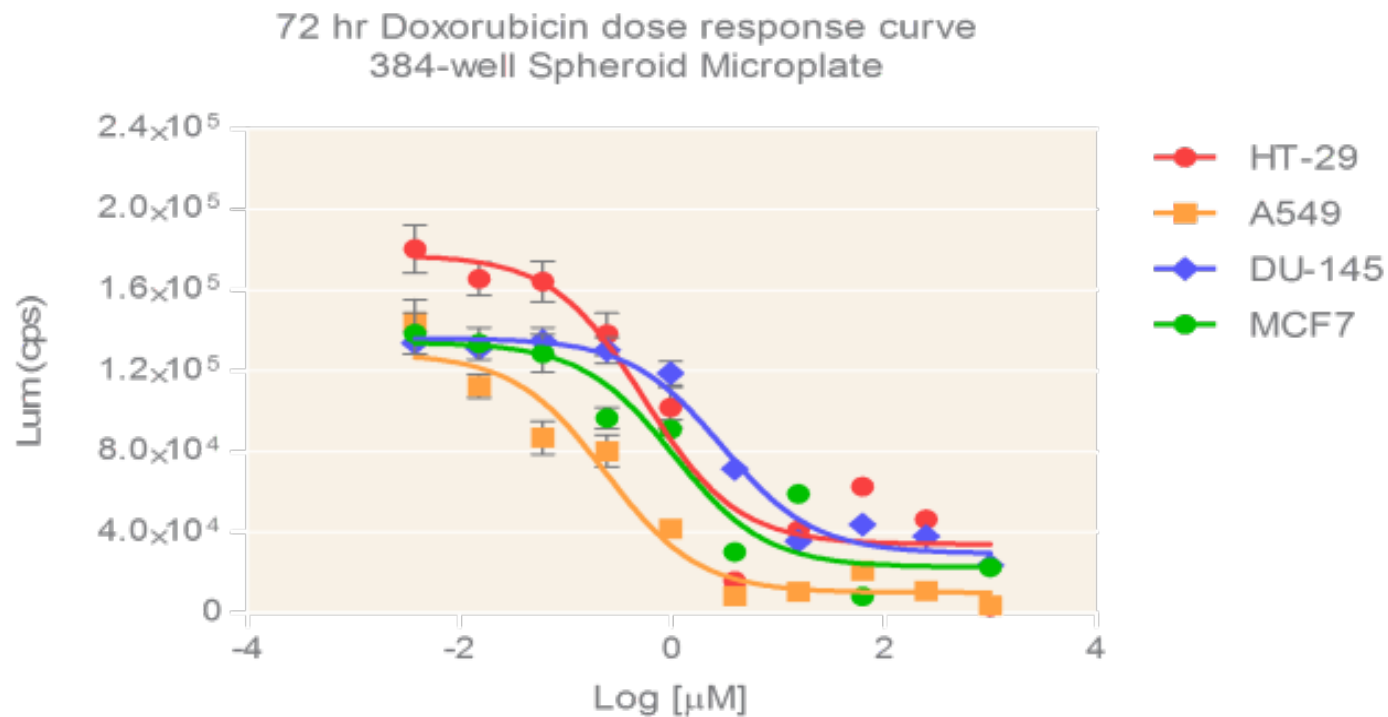


Cisplatin Cytotoxicity



Z-stacked images and corresponding data from DU-145 and Panc-1 spheroids exposed to cisplatin and stained with Hoechst (blue) and propidium iodide (red) to assess cell viability. 10x objective.

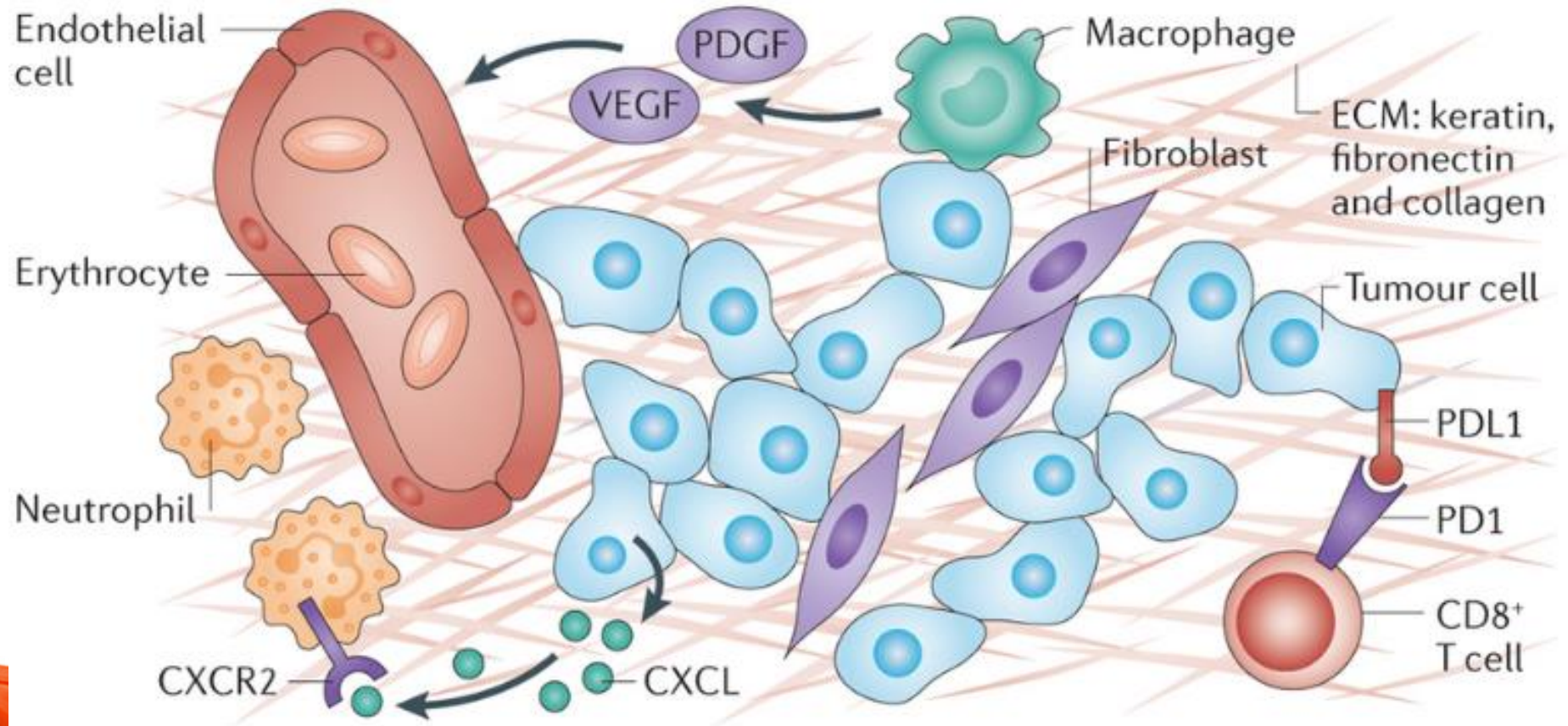
Drug Screening in spheroids using CellTiter-Glo[®] 3D Cell Viability Assay



- Cancer cell lines were plated in 384-well plates at 5K cells/well and allowed to form spheroids for 24 hours.
- Cells were treated with different concentrations of Doxorubicin for 72 hours
- CellTiter-Glo[®] 3D reagent was added (1:1 ratio) into wells to determine cell viability (n = 8)

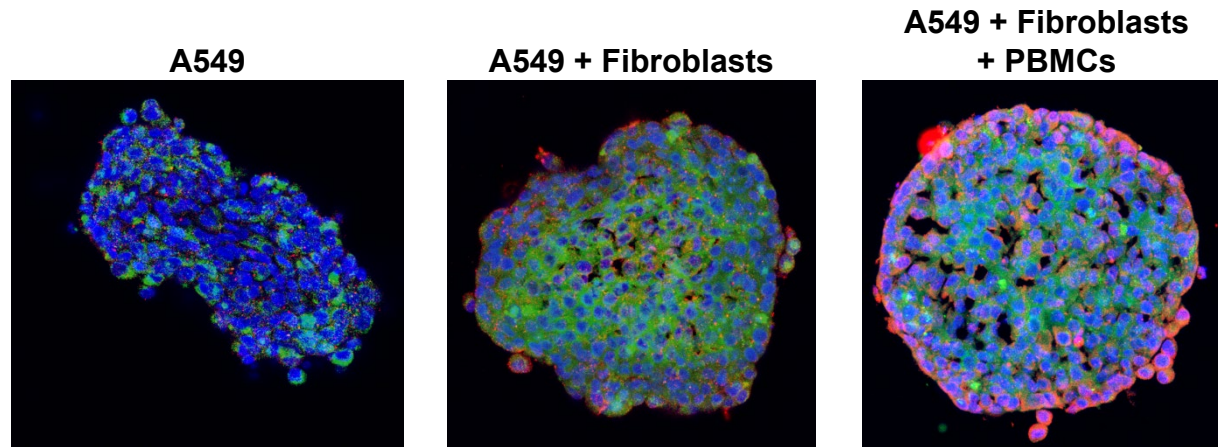
The Tumor Microenvironment Contains Multiple Cell Types

Understanding the complex interactions between cancer cells and other cell types in the tumor microenvironment is important for predicting therapeutic efficacy

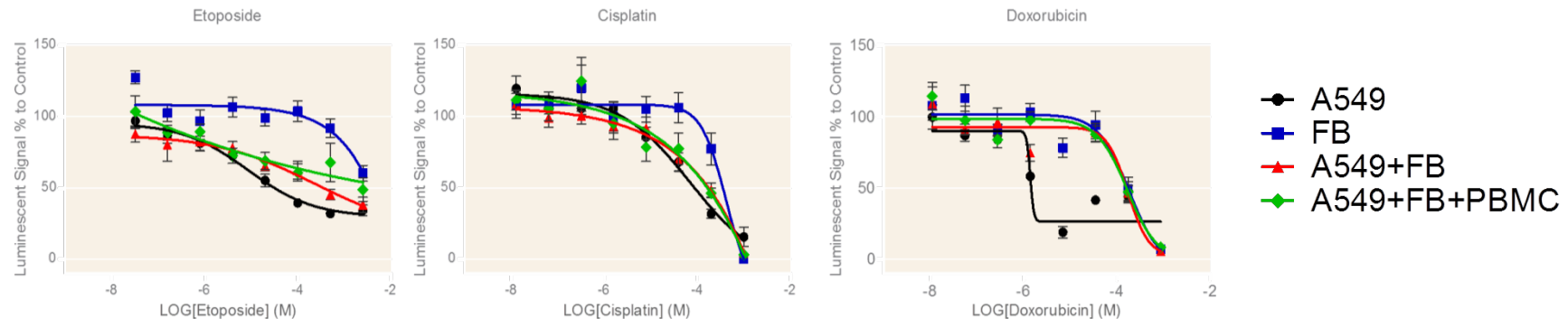


Nature Reviews | Cancer

Co-culture Spheroids can be Formed to Mimic the Tumor Microenvironment with Stromal and Immune cells



A549: Cytokeratin (green)
 Fibroblast: Fibroblast activation protein (red)
 Nuclei: Hoechst (blue)

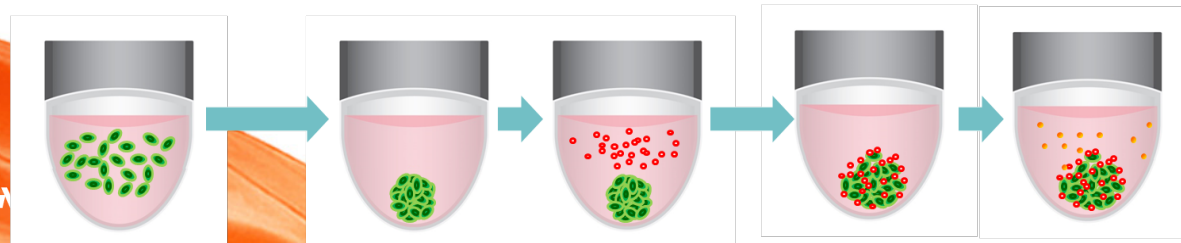
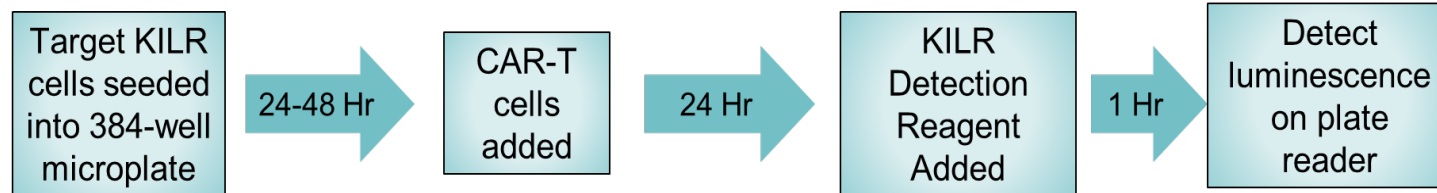
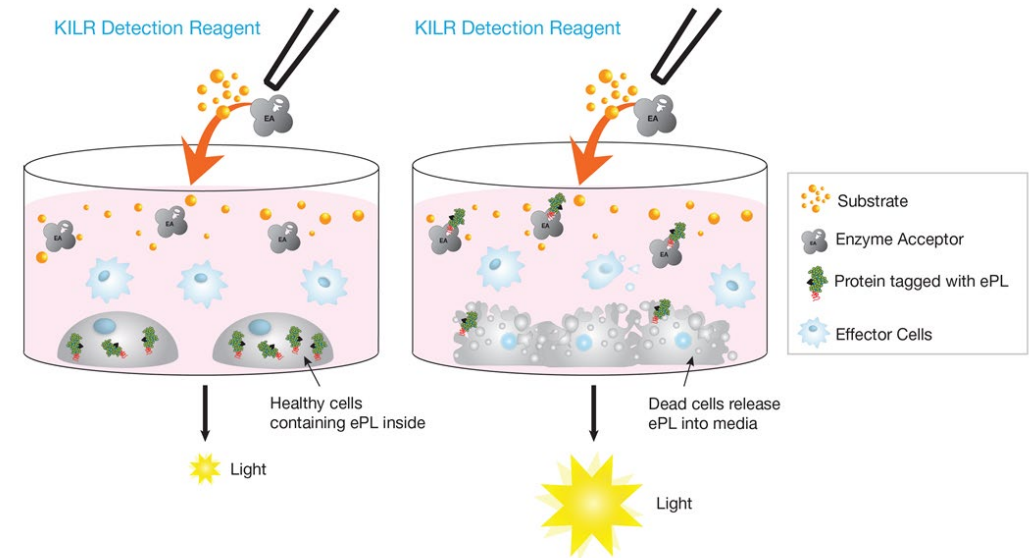
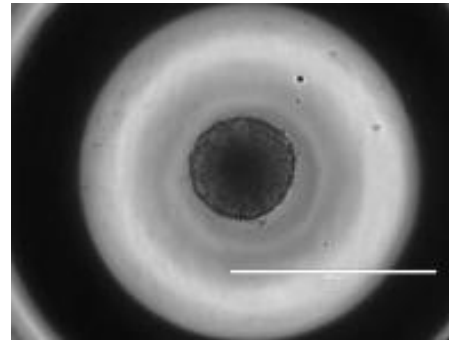


A549 and fibroblasts seeded at 1:9 in a total of 2K cells per well

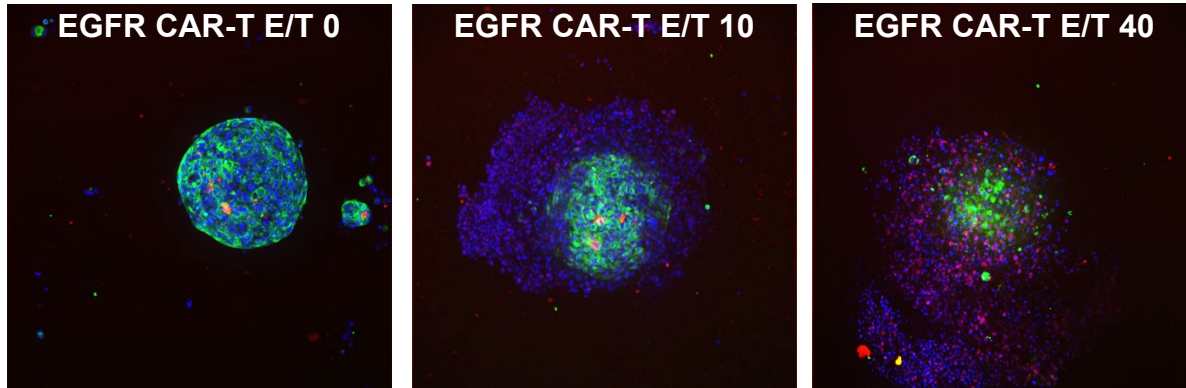
- After 48 hours, peripheral blood mononuclear cells (PBMCs) were added at 1:9 to A549
- Cells were cultured in spheroid microplate for 96 hours prior to fixation (4% PFA) and staining
- Presence of other cell types can affect potency of cancer therapeutics in tumor cell lines

CAR-T Cell Screening in Tumor Spheroids using Corning Spheroid Microplates

In combination with the DiscoverX® KILR® Cytotoxicity Assay, the Corning spheroid microplate provides a high throughput platform for culturing and screening tumor spheroids with CAR-T cell assays.

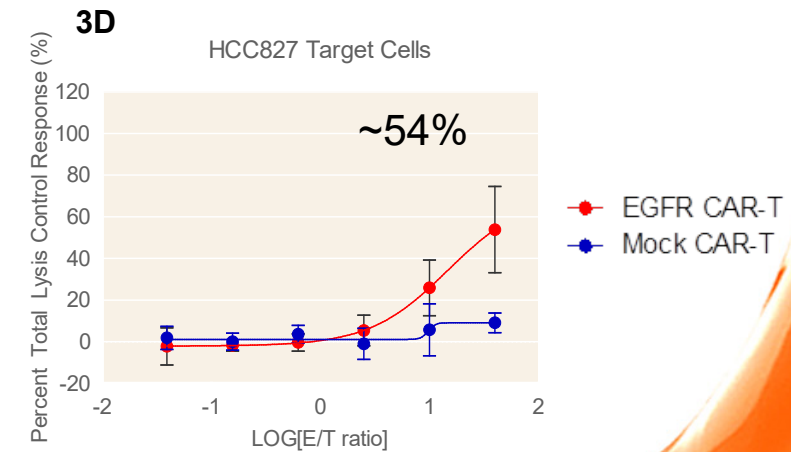
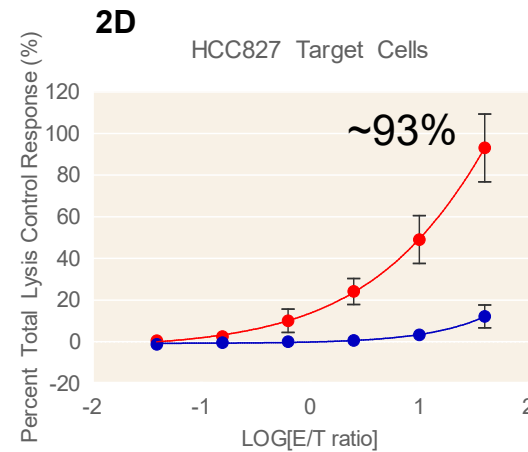
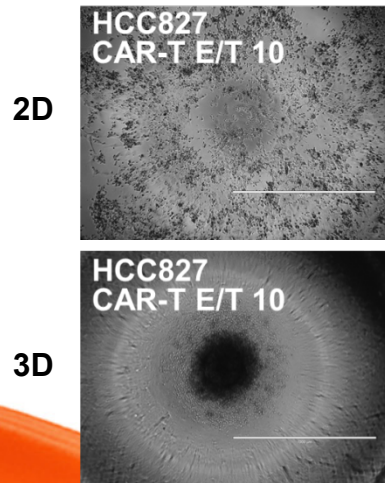


3D Tumor Targeting Assay with CAR-T Cells



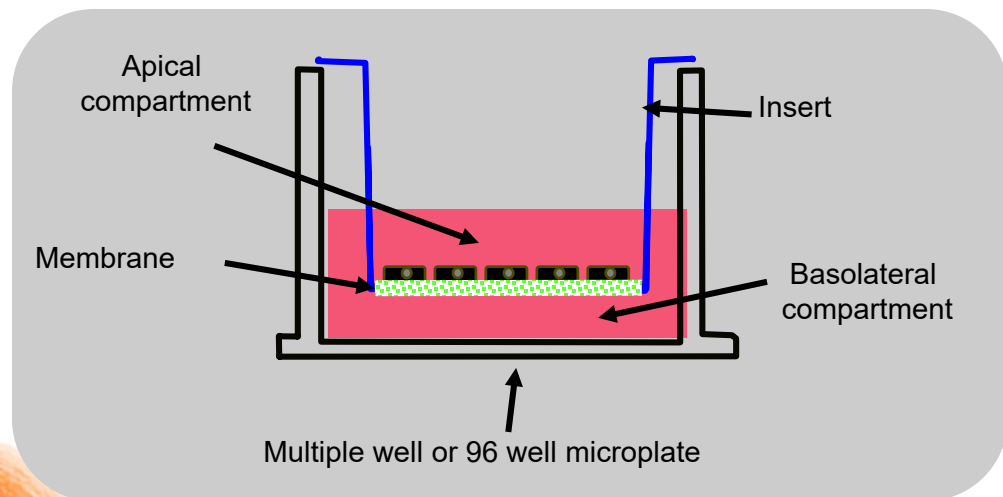
Cytokeratin-7 (green)
 CD3ε (red)
 Hoechst (blue)

24-hours post-CAR-T cell addition to HCC827 tumor spheroids, which contain high EGFR copy number amplifications. Images captured using ThermoFisher CellInsight CX7 in confocal mode with 10X objective.

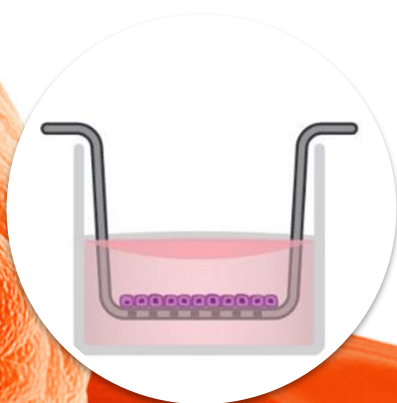


HCC827-KILR cells cultured in 2D and 3D were assayed using KILR detection reagents 24 hours after CAR-T cell addition. N =4.

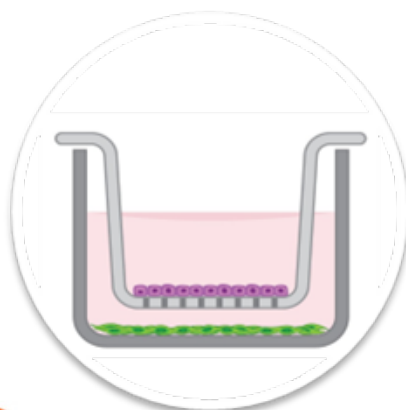
Corning® Permeable Support Systems Provide an *In Vivo*-like Cell Culture Environment



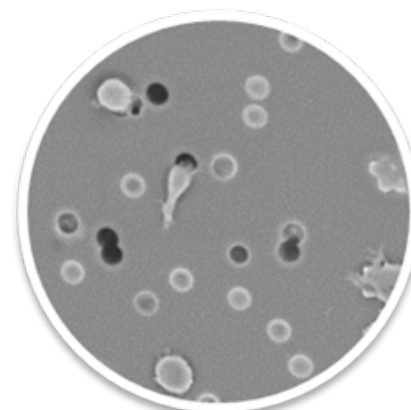
- Different well sizes, membrane materials, and porosity
- Compatible with a range of cell types
- Create physiologically relevant models



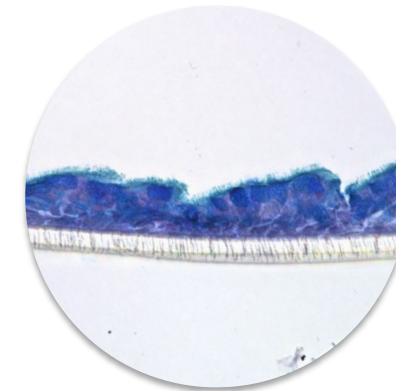
Drug Transport



Co-culture



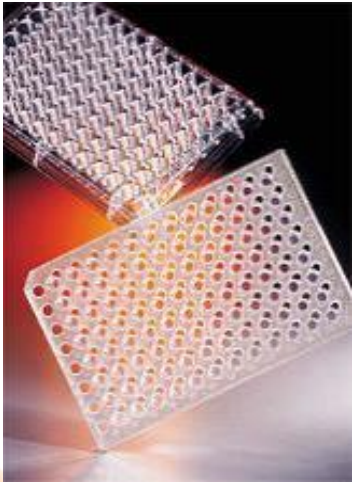
Migration/Invasion



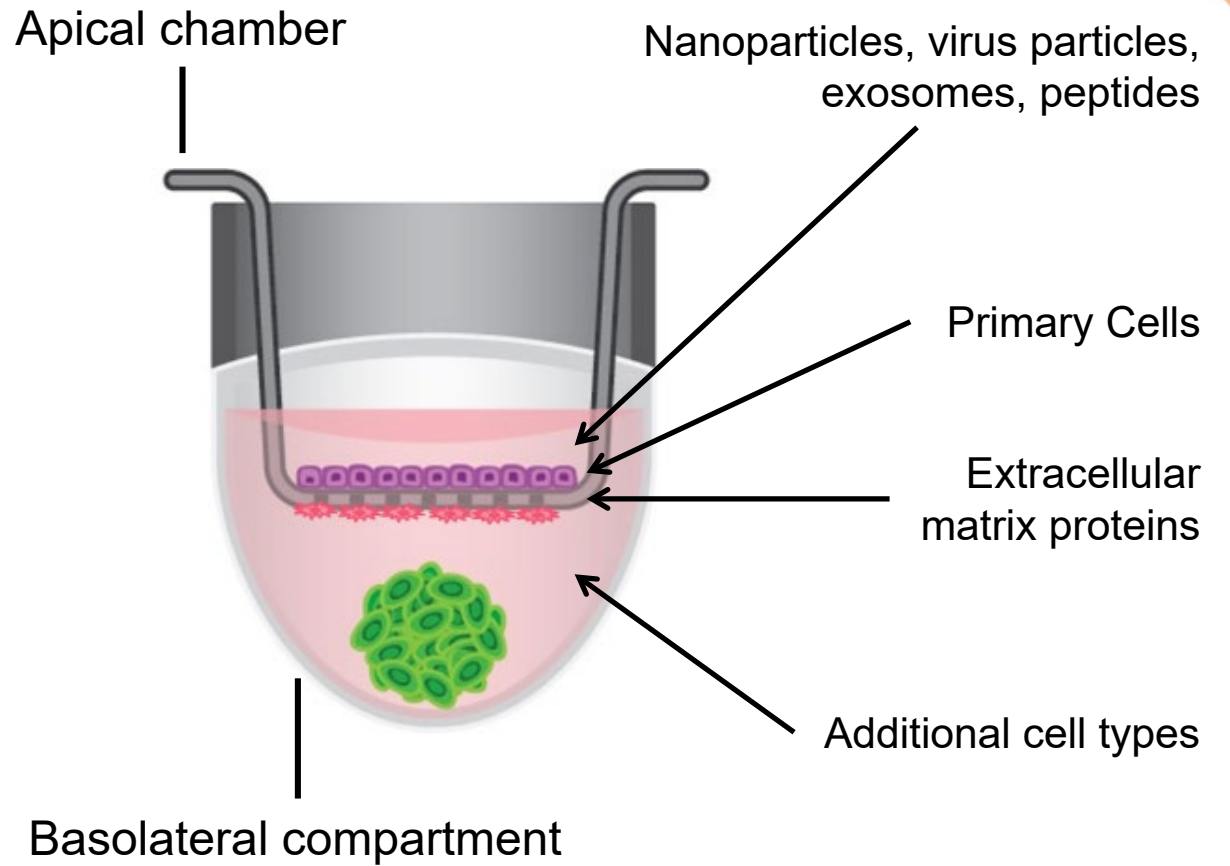
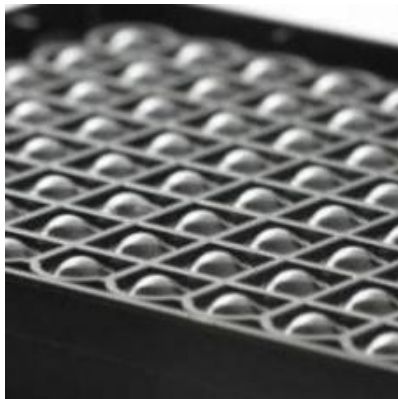
Air-Liquid Interface

Corning Spheroid Microplate/Transwell Combination Models

Corning® HTS-96 Transwell® Permeable Support

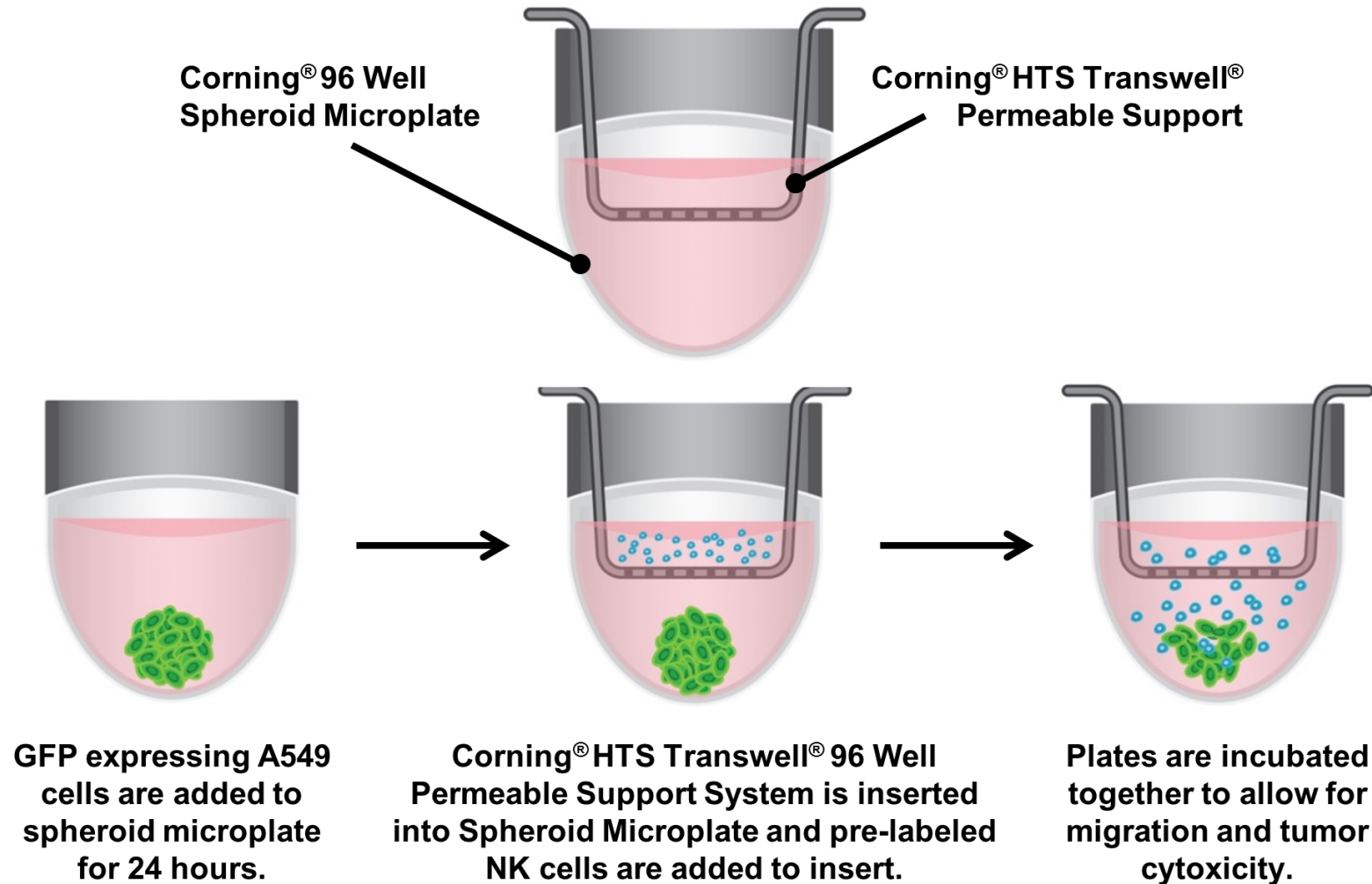


Corning 96-well Spheroid Microplate



3D Immune Oncology Model

NK Cell Migration and Tumor Cytotoxicity



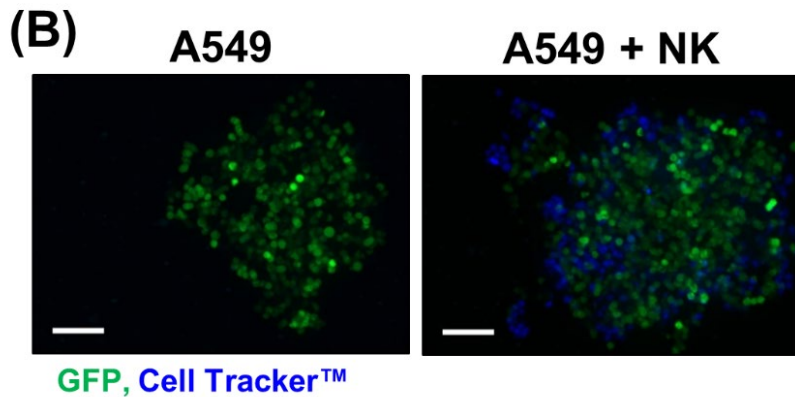
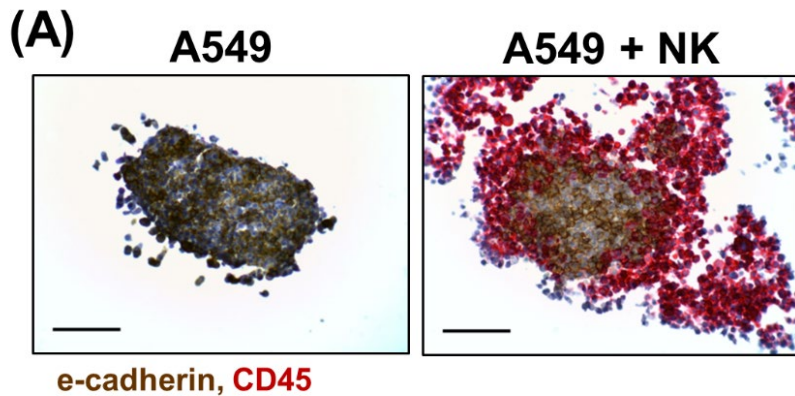
GFP expressing A549 cells are added to spheroid microplate for 24 hours.

Corning® HTS Transwell® 96 Well Permeable Support System is inserted into Spheroid Microplate and pre-labeled NK cells are added to insert.

Plates are incubated together to allow for migration and tumor cytotoxicity.

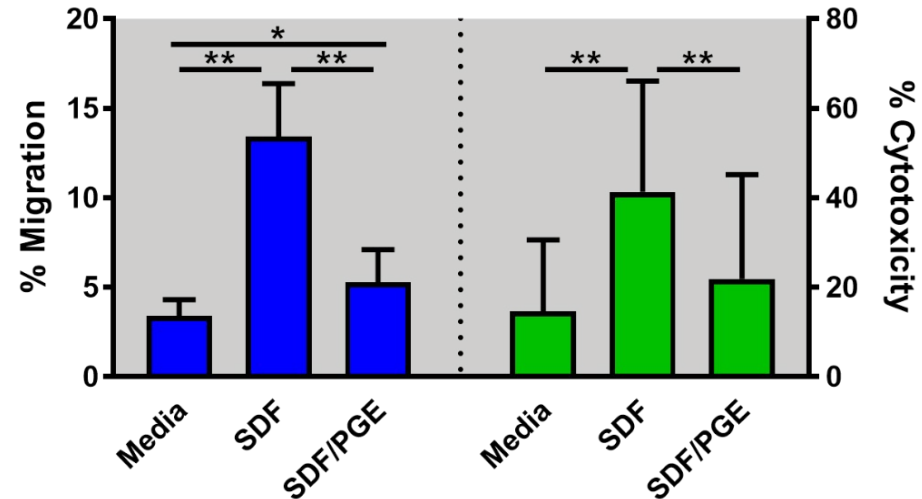
3D Immune Oncology Model

NK Cell Migration and Tumor Cytotoxicity



NK-92MI cell infiltration into A549/GFP tumor spheroids demonstrated in histological sections and confocal imaging (Thermo Scientific CellInsight CX7)

NK-Mediated Cytotoxicity



- NK cell migration/cytotoxicity evaluated in the presence and absence of stromal-cell derived factor-1 α (SDF-1 α ; chemokine) and/or prostaglandin E2 (PGE2) in the medium
- SDF-1 α enhances immune cell migration with highest tumoricidal activity on A549/GFP cells
- PGE blocks migration and cytotoxic effect of NK cells on A549/GFP cells

Summary

- 3D cell culture models offer many advantages over more traditional 2D cell models
- Corning has many tools for helping to achieve 3D cell culture models
- These tools can be combined for more *in vivo*-like models

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Questions?

