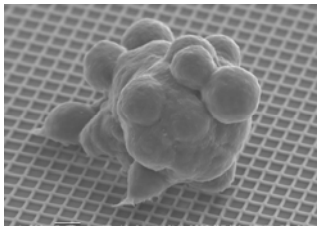
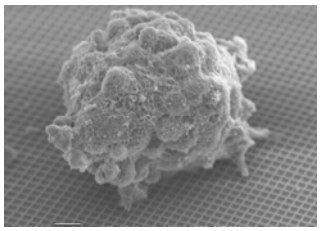
PRODUCE 3D CELL CULTURES WITH CONVENTIONAL 2D TECHNIQUES

SCIVAX Corp.

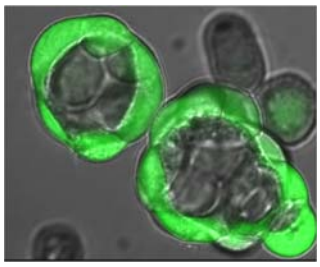


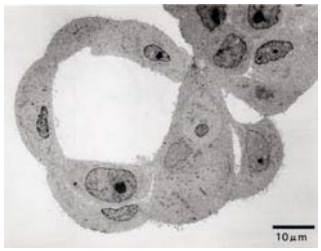


Cells form spheroids

90 types of cultured cells were confirmed to form spheroids on NanoCulture® Plates. Most adherent cell lines form spheroids including cancer lines, primary tumor cells, mesenchymal stem cells and normal primary cells. NanoCulture® Plates are easy to handle and ready to use. Cells are seeded using conventional 2D cell culture techniques, without the need for any gel, matrix or scaffold. The NanoCulture® Plate has a precisely engineered pattern (microsquare or microhoneycomb) on the bottom of the plate that promotes cells to form uniform spheroids. Spheroid morphology is cell line specific ranging from round dense spheres covered with extracellular matrix to spheroids with glandular structure.

SEM image of HeLa cells
Cultured for 2days (top) and 6 days (bottom). The surface of the spheroid (about 100 cells) is covered with the extracellular matrix at day 6. (Courtesy of Dr. Hideo Namiki, Waseda University, Japan)

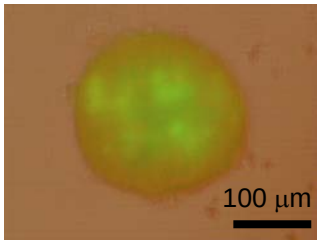




Cell migrate and self-organize into adherent spheroids

On the NanoCulture® Plates cells move, establish cell-cell interactions, and assemble three-dimensionally as spheroids. Smaller spheroids are able to migrate further and merge into larger spheroids. Cells continue to proliferate in the first 7-10 days in the culture and spheroids reach a stable size within 10-14 days. Spheroids may also migrate on NanoCulture® Plates and still stay attached and viable.

MCF7 cells
MCF7 spheroids stained with CalceinAM appear hollow in an confocal microscopy image (top). (Courtesy of Dr. Hideo Namiki, Waseda University, Japan)
The luminal formation has been confirmed by transmission electron microscopy (bottom).

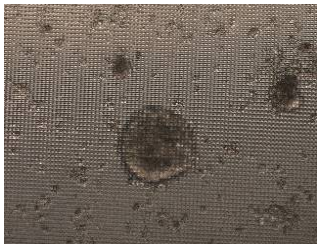


Naturally formed hypoxic areas in spheroids provide a suitable *ex vivo* model for *in vivo* tumors

Hypoxic regions, caused by the rapid proliferation of tumor cells and scarce blood supply, are mostly resistant to radiotherapy and chemotherapy. Tumor cells cultured on the NanoCulture® Plate form spheroids with hypoxic areas in the center. No additional treatment is necessary to induce hypoxia in spheroids.

Hypoxic areas inside the spheroid
HT29, Human Colon Cancer cell line, was transfected with GFP gene under the control of the enhancer motif of HIF(HRE). The transfected cells were cultured on NCP for 7days. The green fluorescence shows the activation of HIF protein. (Courtesy of Dr. Yukie Yoshii, Fukui University, Japan)

Easily culture primary tumors



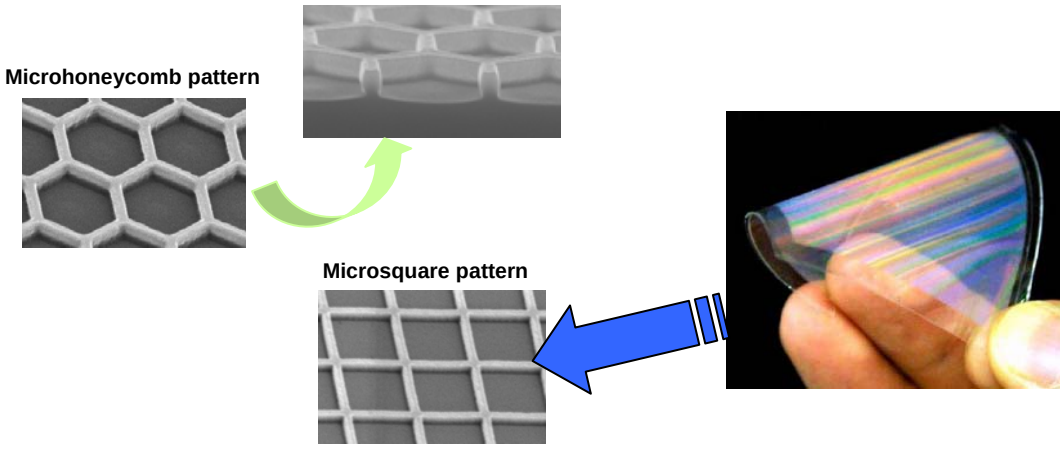
Primary cultured of human pancreatic tumor
Primary tumor cells form spheroids like cancer cell lines.
(Courtesy of Dr. Tetsuya Nakatsura, National Cancer Center East Hospital, Japan)

Primary tumors can also be cultured on the NanoCulture® Plates without overgrowth of fibroblasts. The morphology of the spheroids often resembles the morphology of an *in vivo* tumor. SCIVAX cultured over 100 primary tumor specimens on NanoCulture® Plates in a collaboration with the National Cancer Center East Hospital in Japan. The entire collection of the cultured specimens formed spheroids on the plate. NanoCulture® Plates offer an easy and robust solution to successfully culture virtually any primary tumor.

Unique micro-etched NanoCulture® Plates

NanoCulture® Plate
We offer 96- and 24-well formatted plate.

The synthetic resin film on the bottom of the NanoCulture® Plates contains a micro-scale structure which enables cells to form spheroids without any matrix. The geometry of the pattern is very important for the spheroid growth as cells are able to grasp the ridges of the pattern. Manufacture of the pattern is precisely controlled so there is no lot-to-lot variation. Spheroid formation is uniform and highly reproducible from well-to-well and plate-to-plate.
Most of the cell lines form spheroids on a microhoneycomb and/or a microsquare pattern. We also offer NanoCulture® Media for optimal spheroid growth. Spheroid formation is simple and stable with the right pattern and media combination.



Please see the attached product list.

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NANOCULTURE® PLATES—
A BETTER MODEL FOR DRUG DISCOVERY

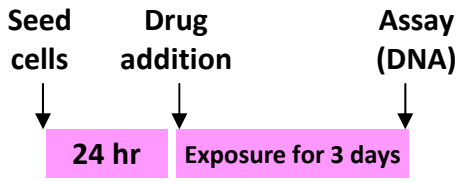
SCIVAX CORP.

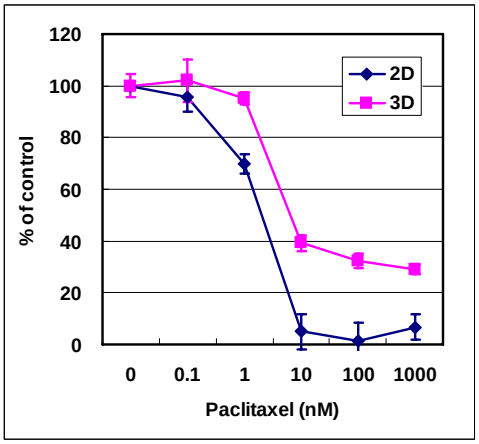
Drug sensitivity studies with NanoCulture® Plates yield substantially different results than those using monolayer culture

Cell based assays for drug discovery screening focus mostly on 2D cell culture. However, tumors in the body are formed as lumps with hypoxic cores and often have different drug permeability and sensitivity compared to a monolayer culture. Spheroids cultured on NanoCulture® Plates represent an advanced model for the drug sensitivity assays. In addition, NanoCulture® Plates provide a solution to evaluate the drug response in a primary tumor specimen.

Paclitaxel

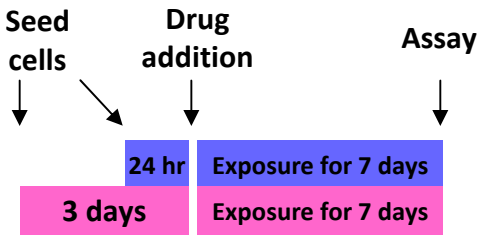
Cell line: BT474 breast cancer
Drug: Paclitaxel

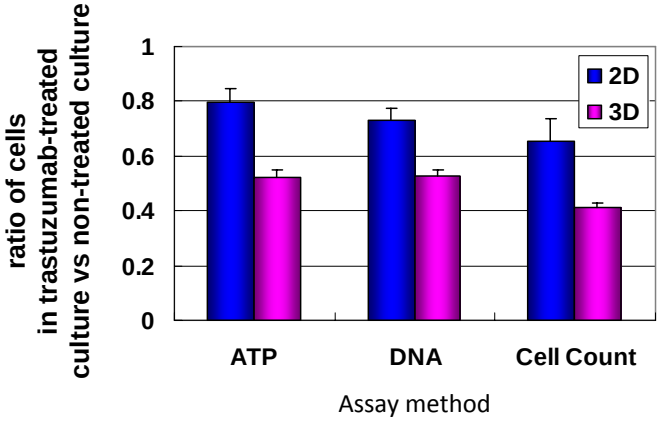




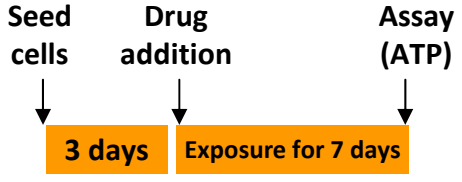
Herceptin® (trastuzumab)

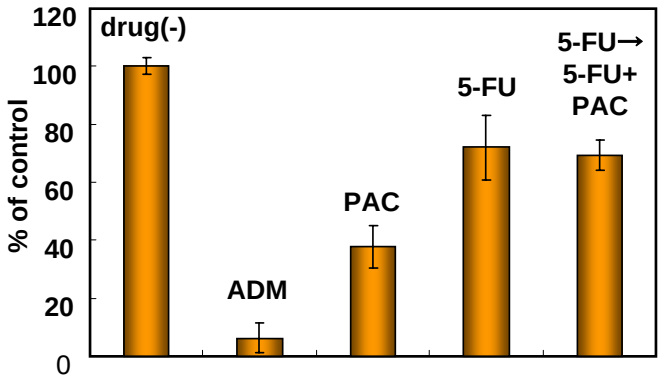
Cell line: BT474
Drug: 10 μg/ ml Herceptin® (trastuzumab)





Primary-cultured human breast cancer spheroid





SPHEROID GENE EXPRESSION PROFILES BETTER MIMIC TUMORS THAN THE SAME CELLS IN MONOLAYER CELLS

HIF-related gene expression is up-regulated in spheroids cultured on NanoCulture® Plates

Tumor cells form spheroids activated with hypoxia-inducible factor 1 (HIF-1) on NanoCulture® Plates. Analysis shows that most genes known to be up regulated with HIF-1 are also up-regulated in these cells.

Analysis of HIF-related gene expression profile in spheroids in NCP:
Comparison between monolayer and spheroids on NCP with DNA microarray analysis

Genes to be known as regulated with HIF-1*		Gene name (Fold change of gene expression)**
Glucose metaboism	Solute carrier family (facilitated glucose transporter)	**Slc2a1(4.7); Slc2a3(5.0)**
	Hexokinase	**Hk2(2.5)**
	Phosphofructokinase	**Pfkfb3 (2.2)**
	Aldolase	**Aldoa (2.7); Aldoc (7.2)**
	Enolase	**Eno2(2.5); Eno3(2.1)**
	Lactate dehydrogenase	**Lhda (2.3)**
	Pyruvate dehydrogenase kinase	**Pdk1 (3.3)**
	LDHA	**Ldha(2.2)**
Promotion of angiogenesis	Angiopoietin-like protein	**Angpt4 (9.2)**
	Vascular endothelial growth factor-related	**Vegfa (6.5)**
	Transforming growth factor-b-related	**Tgfb3 (2.5)**
Vascular tone	Endothelin	**Edn1 (2.1)**
	Adrenomedullin	**Adm (6.8)**
Matrix metabolism	Matrix metallopeptidase	**Mmp1(5.7); Mmp23b(2.5)**
	Serine (or cysteine) peptidase inhibitor	**Serpina3(5.7); Serpina5(2.7); Serpinb3(8.0); Serpinb4(5.3); Serping1(2.5)**
	TIMP metallopeptidase inihibitor	**Timp3(2.1)**
Cell adhesion	Cadherin	**Cdh1(2.5)**
Hypoxia response	Carbonic anhydrase	**Ca9(283); Ca14(2.3)**
Multidrug resistance	ATP-binding cassette (ABC) transporter protein	**Abcc3(3.5); Abcg5(2.7);**
Cell proliferation/survival	Adrenomedullin	**Adm (6.8)**
	Insulin-like growth factor binding protein	**Igfbp3(4.7); Igfbp6(2.7)**
Gap junction	Gap junction protein	**Gjb2(2.7); Gjpb3(2.0)**
Aanti-apoptotic protein	Bcl2/adenovirus E1B interacting protein-related	**Bnip3 (2.6); Bnip3l (3.5)**
Others	Platelet-derived growth factor	**Pdgfb(2.2)**
	Nitric oxide synthase-related	**Nostrin(2.6)**
	Tumor necrosis factor alpha, alpha-induced protein	**Tnfaip3(2.2)**

Data from HCT116 Human colon cancer cells

* Gene selection based mostly on the review from Ke and Costa in Molecular Pharmacology 70; 1469-1480: 2006

**Shows increased expression in spheroids on NCP at Day10 over 2-fold, compared to monolayer at Day3.

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