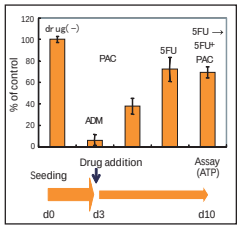
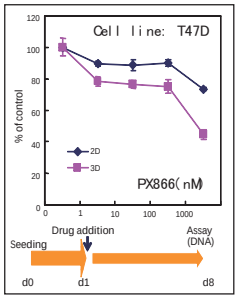
NANOCULTURE®PLATE OFFERS NEW SOLUTION FOR DRUG DISCOVERY

Drug sensitivity studies with the NanoCulture®Plate: Substantially different from monolayer culture

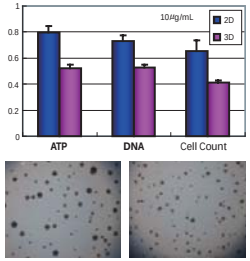
Cell based assays for drug discovery screening focus mostly on 2D cell culture. However, tumors in the body are formed as lumps with hypoxic cores and often have different drug permeability and sensitivity compared to a monolayer culture. Spheroids cultured on the NanoCulture®Plate represent an advanced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



Primary-cultured human breast cancer spheroid
Various anticancer drugs were added after spheroids were formed. The anthracycline drug, adriamycin showed the highest efficacy. (Concentration: Cmax from the clinical use)



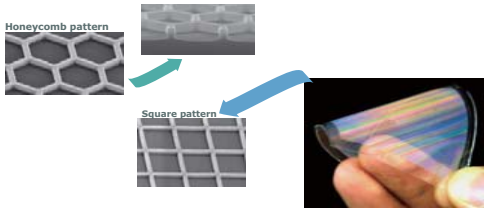
Human breast cancer T47D spheroid
PX866, a PI3K inhibitor, showed higher efficacy in the 3D system compared to the 2D system. (Wortmannin showed the similar results). DNA levels were measured.



Human breast cancer BT474 spheroid
An anti-HER2 antibody drug, Trastuzumab showed high efficacy in the 3D system. ATP level, DNA level, and cell count were measured. Images of the BT474 untreated (left) and treated spheroids are shown in a lower panel.

Features of the NanoCulture®Plate

The thin film on the bottom of the NanoCulture®Plate is covered with a micropattern. The geometry of the pattern is very important for the spheroid growth as cells are able to grab the ridges of the pattern. Manufacture of the pattern is precisely controlled and there is no lot-to-lot variation. Spheroid formation is uniform and highly reproducible: well-to-well and plate-to-plate.
Most of the cell lines form spheroids on a microhoneycomb and/or a microsquare pattern. We also provide NanoCulture®Cell media for optimal spheroid growth. Spheroid formation is simple and stable with the right pattern and media combination.



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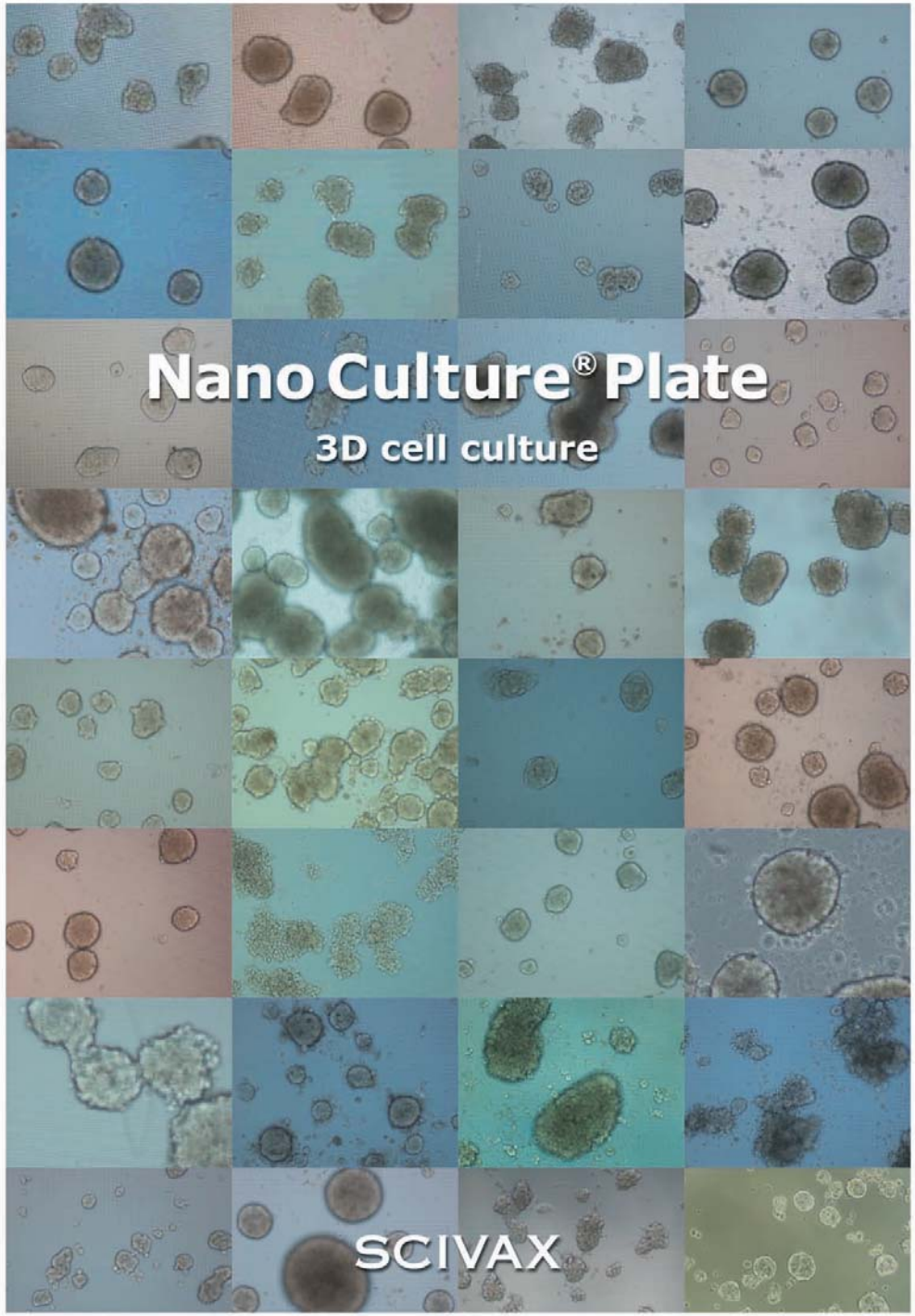
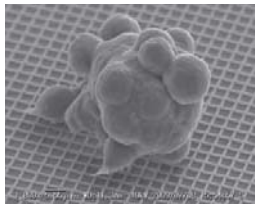
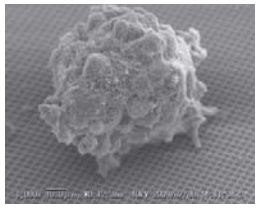
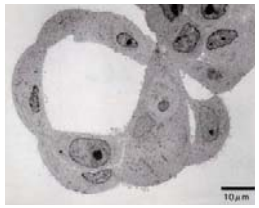


SPHEROIDS ON THE NANOCULTURE® PLATE (NCP) RESEMBLE *IN VIVO* TISSUE MORPHOLOGY AND FUNCTION





SEM image of HeLa cells
Cultured for 2 days (top) and 6 days (bottom). The surface of the spheroid (about 100 cells) is covered with the extracellular matrix at day 6.



MCF7 cells
MCF7 spheroids appear hollow in an inverted microscopy image (top). The luminal formation has been confirmed by transmission electron microscopy of a spheroid cross section (bottom)

Significantly changed pathways (HCT116, Day10)

Diterpenoid biosynthesis
Hedgehog
Lysine degradation
Nucleotide sugars metabolism
Pentose phosphate pathway
Phenylanaline tyrosine and tryptophan biosynthesis
Tetrachloroethene degradation
Ubiquinone biosynthesis

(Analyzed with Gene Spring Ver 10.0)

Most cells form spheroids

The cover page shows diverse forms of spheroids obtained from a variety of cancer cell lines. On the NanoCulture®Plate, most of the adherent cell lines form spheroids including cancer cell lines, primary tumors, mesenchymal stem cells and primary cells.

The NanoCulture®Plate is easy to handle and ready to use. Cells are seeded using the conventional 2D cell culture technique, without the need for any gel, matrix or scaffold. The NanoCulture®Plate has a specific pattern (microsquare or microhoneycomb) on the bottom of the plate that allows uniform spheroid formation. Spheroid morphology is cell line specific ranging from round dense spheres covered with an extracellular matrix to spheroids with a glandular

Cells migrate to form spheroids on the NanoCulture®Plate: Spheroids are self-organized and adherent

On the NanoCulture®Plate cells move, establish cell-cell interactions and assemble 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 can migrate on the NanoCulture®Plate and still stay attached and viable.

Differential Gene Expression: 3D versus 2D cell culture - Over 2500 Genes are changed -

Morphological changes correlate with the alteration of gene expression in spheroids on the NanoCulture®Plate compared to a monolayer culture. As expected, VEGF is one of the genes whose expression increased in spheroids. Gene expression of over 2,500 genes has been changed in spheroid culture either by over 2 fold increase or less than a half fold decrease.

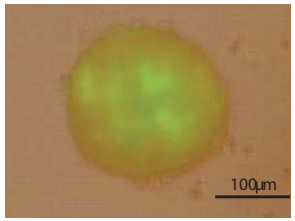
Representative changes in gene expression profile of HCT116 cells, cultured for 10 days on the NanoCulture®Plate.

Increased	Decreased
Hexokinase (2.4)	FGF2 (0.13)
E-Cadherin (2.5)	TERT (0.36)
Interferon α inducible protein27 (6.3)	MDR (0.16)
Transferin (2.9)	BAX (0.28)
Jun oncogene (3.9)	CDC2 (0.47)
EGF-R (2.6)	Centromere A~P (0.1~0.3)
VEGF (6.5)	Cyclin A2 (0.26)
Caspace 4 (2.6)	DHFR (0.4)
Caspace5 (3.0)	E2F (0.46)
etc... > 2 fold in 1450 genes	etc... < 0.5 fold in 1350 genes

(Changes are analyzed with Microarray analysis, n=2)

Hypoxic area naturally forms in spheroids on the NanoCulture®Plate -The most 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)

Primary Tumor Culture

Primary tumors can also be cultured on the NanoCulture®Plate. The morphology of the spheroids often resembles the morphology of an *in vivo* tumor. SCIVAX cultured over 100 primary tumor specimens on the NanoCulture®Plate in a collaboration with the National Cancer Center East in Japan. The entire collection of the cultured specimens formed spheroids on the plate.

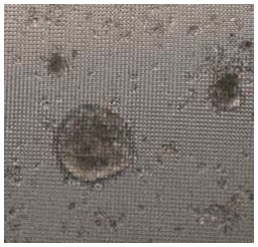
The NanoCulture®Plate offers an easy and robust solution for primary tumor culture with a high success rate.

Enhanced spheroid differentiation and function on the NanoCulture®Plate

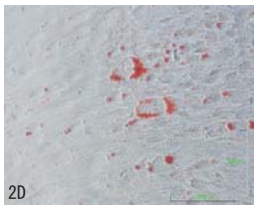
Human stem cells (such as mesenchymal stem cells) grown as spheroids on the NanoCulture®Plate exhibit superior differentiation capability compared to those cultured in a monolayer.

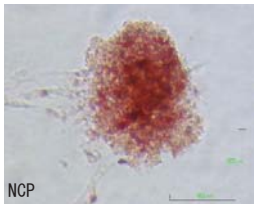
During the spheroid formation cells establish tight cell-cell interactions which allow cells to differentiate at faster rates than cells in a 2D cell culture. Expression of certain genes (such as those for cytokines and metabolic enzymes) is enhanced in spheroids on the NanoCulture®Plate as well.

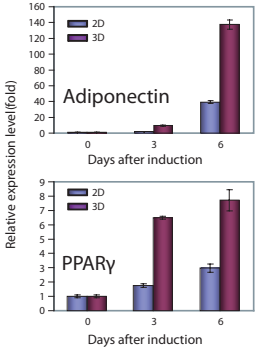
The NanoCulture®Plate allows culture of highly functional spheroids with *in vivo* -like characteristics.



Primary cultured of human pancreatic tumor
Spheroids with a glandular structure are shown. This phenomenon is often observed in primary culture of pancreatic tumors on the NCP.







Mesenchymal stem cells UET-13
These cells can differentiate into round mature adipocytes in a short period of time on the NanoCulture Plate. A large single lipid droplet is sometimes observed on the NanoCulture Plate.