Product list

Code	Product	Content
NCP-LS96-3	NanoCulture® Plate (NCP), Low-Binding Microsquare, 96 wells	3 plates
NCP-LS96-10		10 plates
NCP-LS24-3	NanoCulture® Plate (NCP), Low-Binding, Microsquare, 24 wells	3 plates
NCP-LS24-10		10 plates
NCP-HS96-3	NanoCulture® Plate (NCP), High-Binding, Microsquare, 96 wells	3 plates
NCP-HS96-10		10 plates
NCP-HS24-3	NanoCulture® Plate (NCP), High-Binding, Microsquare, 24 wells	3 plates
NCP-HS24-10		10 plates
NCP-LH96-3	NanoCulture® Plate (NCP), Low-Binding, Microhoneycomb, 96 wells	3 plates
NCP-LH96-10		10 plates
NCP-LH24-3	NanoCulture® Plate (NCP), Low-Binding, Microhoneycomb, 24 wells	3 plates
NCP-LH24-10		10 plates
NCP-HH96-3	NanoCulture® Plate (NCP), High-Binding, Microhoneycomb, 96 wells	3 plates
NCP-HH96-10		10 plates
NCP-HH24-3	NanoCulture® Plate (NCP), High-Binding, Microhoneycomb, 24 wells	3 plates
NCP-HH24-10		10 plates
NCP-LSH96-2	NanoCulture® Plate (NCP), Low-Binding, Microsquare/ Microhoneycomb, 96 well	1 plate each (2 plates)
NCP-LSH24-2	NanoCulture® Plate (NCP), Low-Binding, Microsquare/ Micorohoneycomb, 24 well	1 plate each (2 plates)
SD4X	Spheroid Dispersion Solution (4X)	15ml
SLB	Spheroid Lysis Buffer	7.5ml×2
NCM-M100	NanoCulture® Medium M-type	50ml×2
NCM-M200		50ml×4
NCM-R100	NanoCulture® Medium R-type	50ml×2
NCM-R200		50ml×4

SCIVAX CORP.
Japan
Kanagawa Science Park E502
3-2-1 Sakado, Takatsu-ku, Kawasaki-shi
Kanagawa 213-0012 Japan

Phone:+81-44-820-0551
Fax:+81-44-820-0552
E-mail: cell@scivax.com
http://www.scivax.com/

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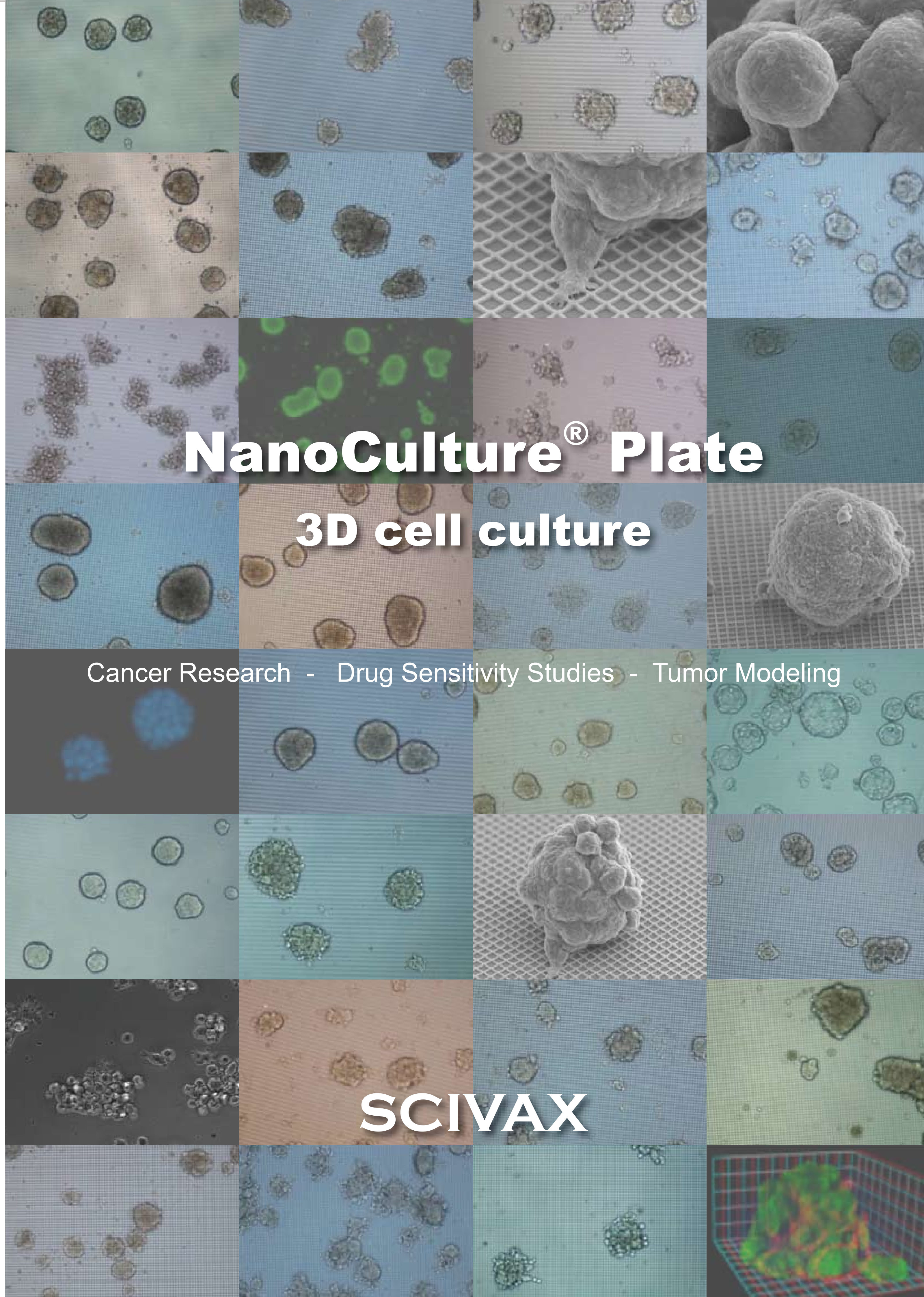


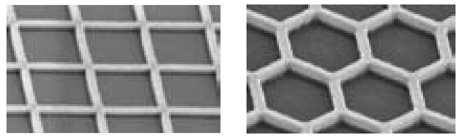
Cell spheroid formation by modulating cell adhesion and motility

Overview of NanoCulture® Plates

Produce 3D cell cultures using conventional 2D techniques with NanoCulture® Plates

Each NCP® is engineered with a micro-patterned square or honeycomb pattern that encourages 3D cell culture growth. Each plate is easy to handle and ready to use; cells are simply seeded using conventional 2D culture techniques and form spheroids as they migrate along the plate's low-binding micro-patterned pattern. Precise engineering of the patterns results in nearly zero well-to-well and lot-to-lot variability, a feature of NCP® that cannot be achieved by either gel or matrix-based cell culture.

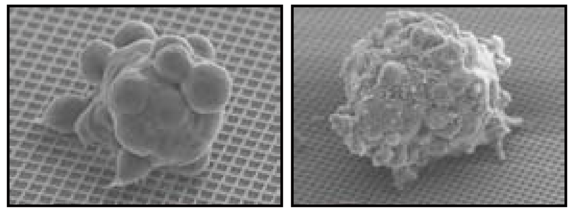
NanoCulture® Plate



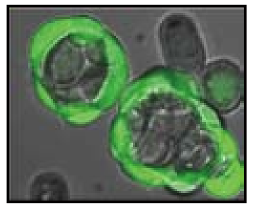
NCP® microsquare and microhoneycomb patterning

Similar spheroid morphology *in vivo* and *in vitro* on NCP®

Over 90 types of cells form spheroids on NCP®, including cancerous cells, primary tumor cells, mesenchymal stem cells, and non-cancerous cells. For example, dense HeLa spheroids producing high levels of extra cellular matrix (ECM) and high polarity cells with tuberous spheroids were both empirically observed on NCP®.



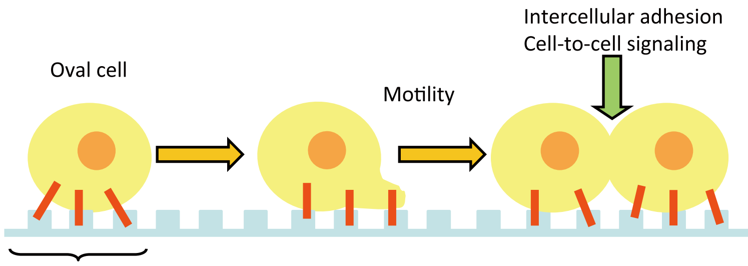
SEM images of a HeLa cell spheroid on NCP® 2 days after seeding (left) and 6 days after seeding (right)
Note that the spheroid's surface is covered with ECM
Data provided by Dr. H. Namiki, Waseda University



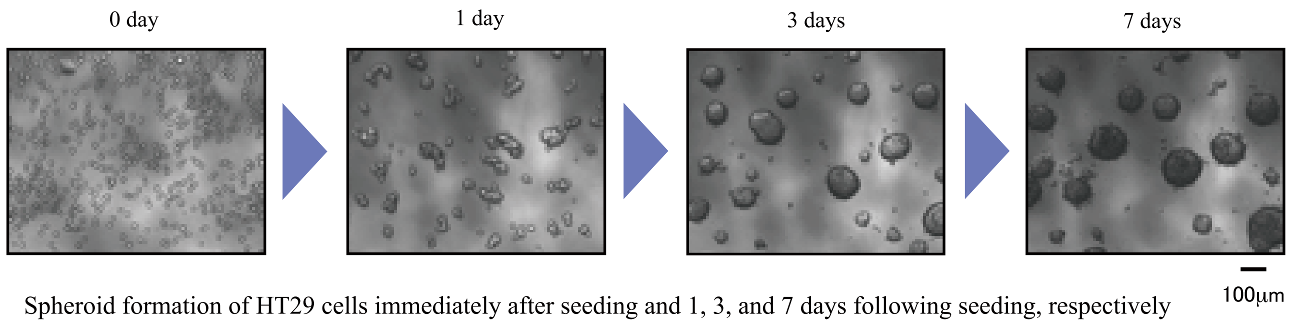
Confocal microscopy of MCF7 cells
Live cells stained with Calcein AM 3 days after seeding on NCP®
Data provided by Dr. H. Namiki, Waseda University

NCP® spheroid formation

The precise engineering behind the low-binding NCP® allows cells to move, establish cell-cell interactions, and assemble into three-dimensional spheroids. Spheroid adhesion plays a vital role in ensuring cell viability and successful spheroid formation.



Mechanisms of NCP® spher oid formation



Spheroid formation of HT29 cells immediately after seeding and 1, 3, and 7 days following seeding, respectively

Grow functional cells with NCP®

Variety of NCP® spheroid functions

In vivo-like gene expression

Spheroid morphology and gene expression are highly correlated. However, this is challenging to study using conventional 2D cell culture. For example, the level of vascular endothelial growth factor (VEGF) expression in a tumor mass can only be observed *in vitro* when a three-dimensional spheroid is formed. Spheroids formed on NCP® are ideal for investigating changes in gene expression.

Observed pathways significantly different from 2D culturing after 10 days of spheroid formulation on NCP® using HCT1 16 cells.

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

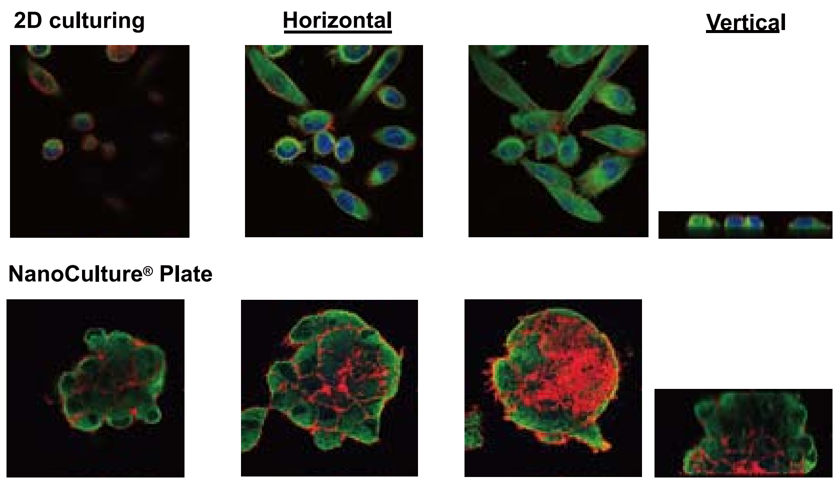
（Analysed by Gene Spring ver.10.0)

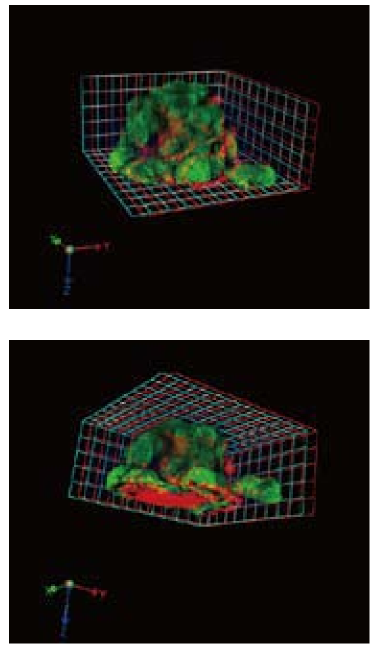
Genetic generation of the spheroids of HCT116 cells (10 days) compared with 2D cell culture systems.

Increased on NCP®	Decreased on NCP®
Hexokinase (2.4)	FGF2 (0.13)
E-Cadherin (2.5)	TERT (0.36)
Interferon α inducible protein27 (6.3)	MDR (0.16)
Transferrin (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)
Caspase 4 (2.6)	DHFR (0.4)
Caspase 5 (3.0)	E2F (0.46)
1450 types of gene were doubled or more	1350 types of gene were half or lower

Isolating spheroids from NCP®

Spheroids grown on NCP® can be easily removed from the plate for use in subsequent studies. This unique benefit of NCP® is not a feature of other commercially available gel and matrix 3D cell culture systems. Further, the orientation of proteins within spheroids formed on NCP® can be easily observed using a variety of immunological stains, again a feature that is not currently possible with other available 3D culture methods.





3D images of spheroids
Above: horizontal view
Bottom: view from bottom

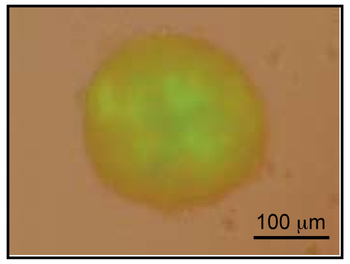
Fluorescent immunostaining of PANC-1 cell spheroids grown on NCP®
All cells were cultured in 2D on a glass plate or in 3D using NCP®. Following a 5-day incubation period, cells were fluorescently imunostained for α-tubulin, F-actin and DNA and then observed using fluorescence confocal microscopy (FCM). Vertical and spectroscopic images were obtained with image analysis software; observe the surface and intracellular F-actin orientation.
Green: α -tubulin, Red: F-actin, Blue: DNA stained with DAPI
Data provided by Professor Yoko Matsuda, Nippon Medical School

NCP® Applications

Tumor modeling

Natural formation of hypoxic regions within spheroids

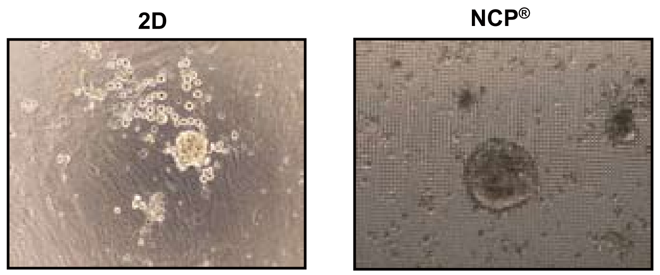
In vivo, tumors exhibit hypoxic regions caused by the rapid proliferation of cells and scarce blood supply that are mostly resistant to radiation and chemotherapy. Spheroids from NCP® also exhibit naturally formed hypoxic regions and, therefore, similar metabolic pathways *in vivo* tumor cells, making NCP® spheroids a suitable *ex vivo* model for *in vivo* tumors.



Hypoxic regions within spheroids
After 7 days of incubation on NCP using cells with GFP vectors (HIF enhanced). Activation of HIF can be observed inside the spheroids.
Data provided from Dr. Yukie Yoshii of Fukui University.

Primary tumor are easily cultured on NCP®

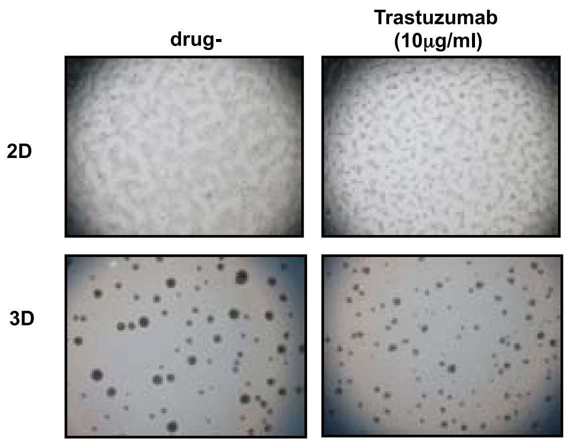
Cancer cells grow alongside interstitial cells *in vivo*; however, the overgrowth of fibroblasts disrupts the growth of primary tumor cells in conventional 2D culture. NCP® spheroids can be cultured without the overgrowth of interstitial cells and often share morphologies with *in vivo* tumors; SCIVAX has successfully cultured over 100 types of primary tumor cells in collaboration with the Japanese National Cancer Research Center. NCP® is an easy and robust solution to primary tumor culture.

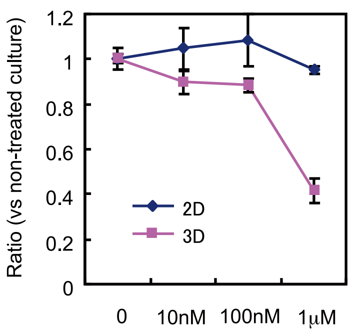


Initial incubation of human pancreatic cancer cells
Spheroids formation is observed only on NCP (right) but not on 2D plate (left). Proliferation of interstitial cell seemed to be controlled on NCP culturing.
Data provided from Dr Tetsuya Nakatsura of National Cancer Center.

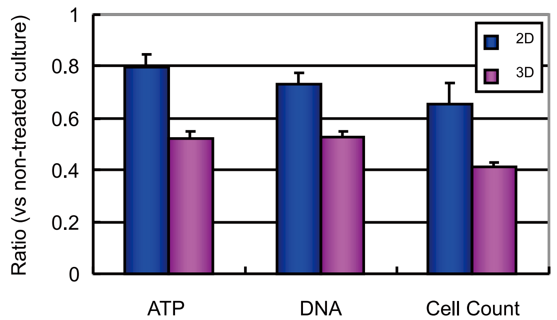
Anticancer drug sensitivity

Drug sensitivity studies show that responses to anticancer drug differ substantially in 2D cell culture versus 3D NCP® cell culture. Spheroids grown on NCP® are morphologically and metabolically similar to *in vivo* tumors, thus offering an advanced model for drug sensitivity assays and drug response evaluation in primary tumors.





HT29 human colorectal cancer cell line
Higher drug efficiency observed for cells cultured with NCP® than 2D cultured cells when tested for Radicicol (HSP90 blocker)

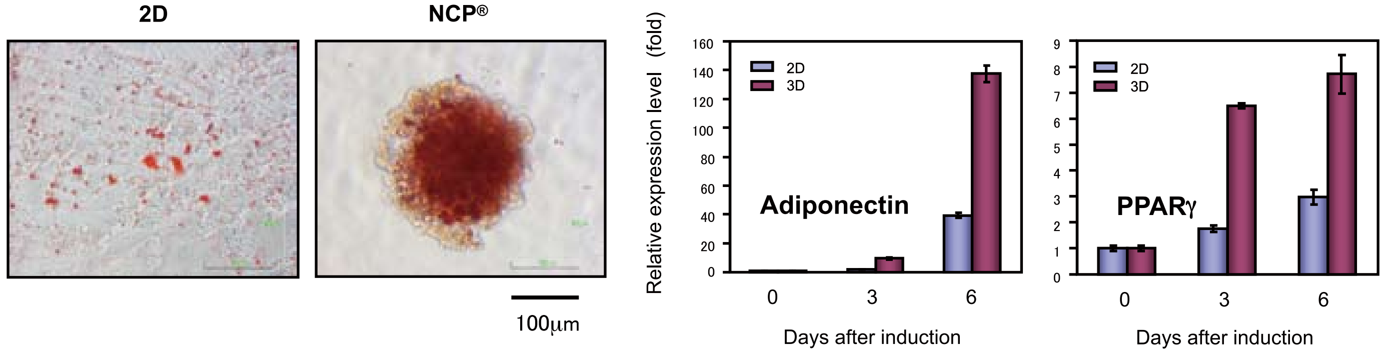


BT474 human breast cancer cell line
Microscopic images and drug sensitivity assays comparing 2D cell culture and NCP® using HER2 blocking drug Trastuzumab. In each dimension analyzed, higher drug efficiency was observed in NCP® spheroids. Further, Trastuzumab-treated NCP® spheroids had depressed cell counts and decreased levels of both DNA and ATP compared to treated and cultured 2D cells. Photo: Left: Control (no drug), Right: With Trastuzumab

Regular cells form spheroids on NCP®

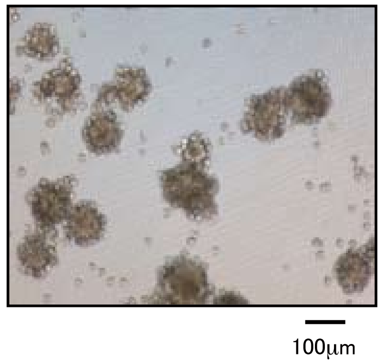
In addition to cancer cells, regular cells also form spheroids on NCP® . Intercellular networks and surface adhesion increase with spheroid formation, resulting in increased cell differentiation potency and differential expression of metabolic enzymes and cytokines. Unlike cancer cells, regular cells grown on NCP® stop expanding following spheroid formation. NCP® can overturn our classic understanding- now *in vitro* can be close to *in vivo*!

Stem cells

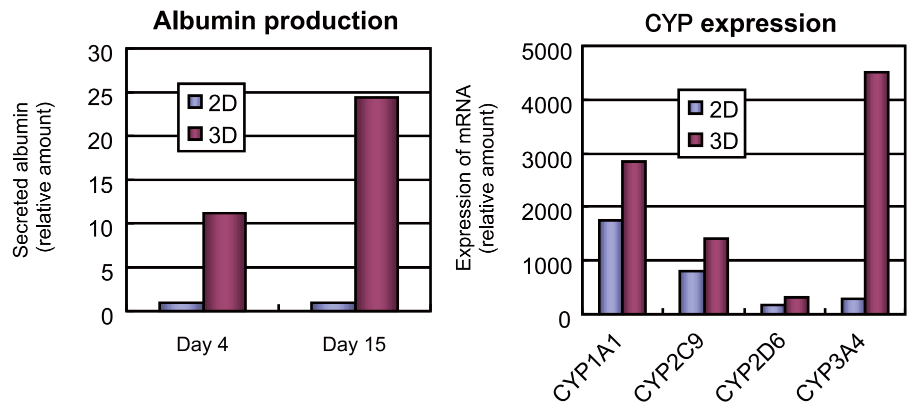


Adipose differentiation of human mesenchymal stem cells (UET-13)
Fat cell spheroids is observed after incubating human mesenchymal stem cells (UET-13) on NCP®.
Data provided by Dr Hajime Okida of National Research Institute for Child Health and Development

Liver cells



Spheroid of primary rat hepatocyte
Hepatocytes obtained from rat liver forms spheroids on NCP®.

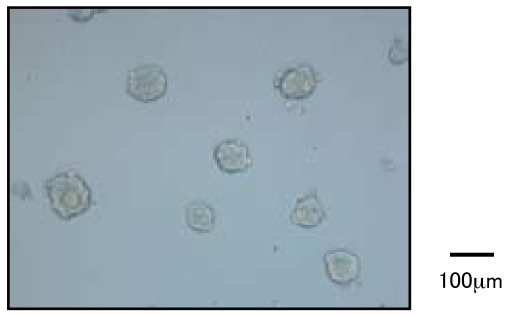


Functional expression of immortalized human hepatocytes
Higher level of albumin production and mRNA of CYP expression on NCP® compared with 2D culturing.

Cardiac mussel

Spheroid of primary rat cardiac cell
Cardiac cells obtained from rat heart forms spheroids on NCP® and start pulsating.

Fibroblast



Spheroid of human lung derived fibroblast – VA-13
High motility of fibroblast on NCP® surface to form spheroids. No significant proliferation like cancer cells.

Benefits of NanoCulture® Plates

100% synthetic, no biomaterials
- Nano-scaled patterning with synthetic polymer
- Reliable product exhibits no lot-to-lot variability
- Matrix-free, gel-free
- High transparency for clearer observation

Simple halindng – just inoculate cells on NanoCulture® Plates to get spheroids
- Over 90 types of cells produce spheroids
- No well-to-well differences
- High spheroid reproducibility
- Grow cancer cells alongside interstitial cells

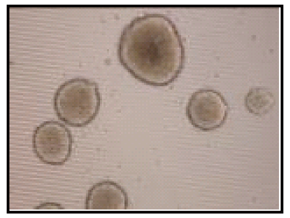
Matrix-free, low-binding substrate
- High cell viability
- Easy isolation of the spheroid

Type of cells forming spheroids on NCP®:

Cancer cells of strain	NIH/3T3	Preadipocyte	Glial cell	Mouse ES cells
Primary cancer cells	3T3-L1	Osteoblast	Vascular endothelial cells	Others
Fibroblast	Mesenchymal stem cells	Cardiomyocyte	Synoviocyte	
Hepatocyte	Preadipocyte	Neuron	MEF	

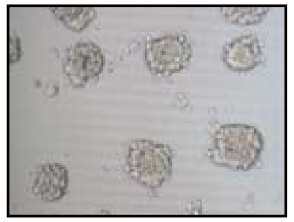
Types of spheroids

Oval spheroids with flat surface



(e.g. HCT116)

Rough surface spheroids



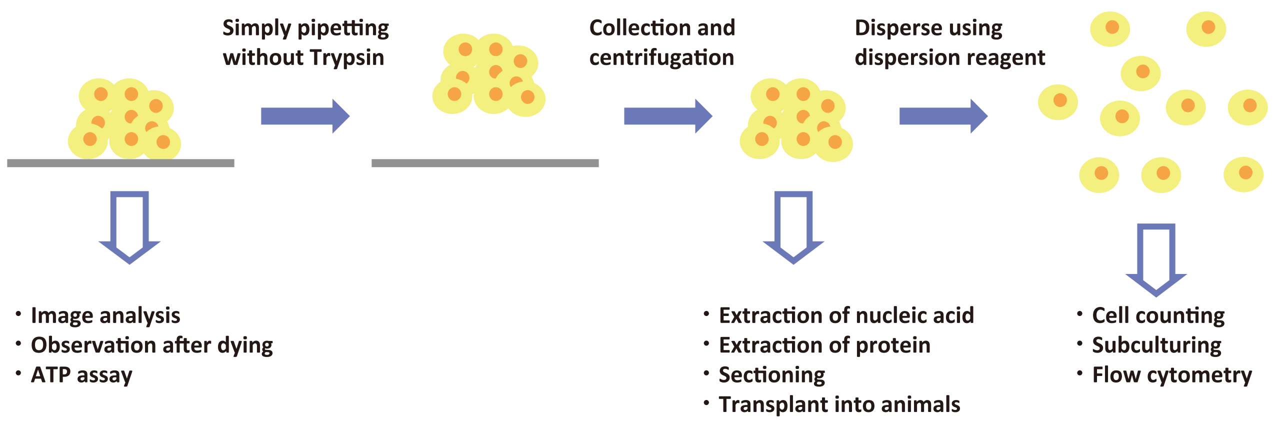
(e.g. PC-3)

Grape-like structure

(e.g. SK-BR3)

Optimization to obtain spheroids at the best condition is required as preliminary study.

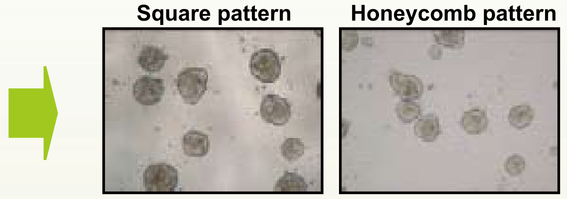
Subsequent usage of obtained spheroids



FAQ

Q1. Which type of NCP® should I use?

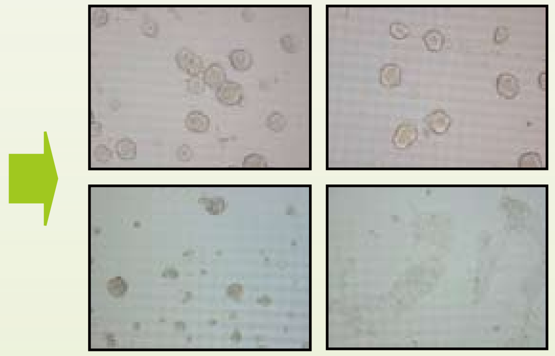
A. NanoCulture® Plates come in two micro-etched patterns, either a microsquare or microhoneycomb pattern. Cell lines exhibit different spheroid morphology; please ask Scivax technical service or test both as your preliminary study.



Example of better spheroid formulation using square patten

Q2. Should I use a low adhesion or high adhesion NCP® ?

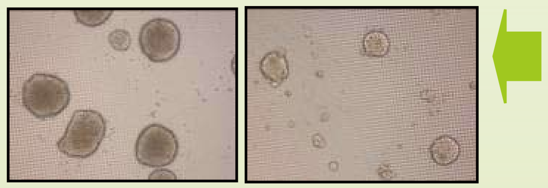
A. Scivax recommends beginning with the low adhesion plate. If you find that spheroids adhere poorly to the substrate under those conditions, please try the high adhesion plate. Please note that 3D spheroids cannot form when adhesion is too strong and, instead, will behave similarly to monolayer cultures.



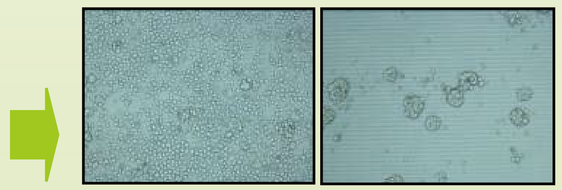
Above: Better adhesion using high adhesion type
Bottom: Better adhesion using lower adhesion type

Q3. Under what conditions should I use the M-type and R-type media?

A. Our M-type media is specifically designed to obtain cancer cell spheroids. If spheroids are not formed using M-type media, our R-type media can be used to encourage spheroid formation. Please ask Scivax technical service for details.



Better spheroid formulation using M type medium



Better spheroid formulation using R type medium

Q4. What is the difference between the Spheroid Dispersion Reagent and Spheroid Lysis Buffer?

A. The Spheroid Dispersion Solution helps segment spheroids in to individual cells, whereas the Lysis buffer lyses individual cells so their contents can be used for further experimentation, such as DNA analysis.