

# 3D tumor spheroids

This manuscript ([permalink](#)) was automatically generated from [SharikHR/review@c7d5a81](#) on May 8, 2020.

## Authors

---

- **Sharik Hernandez**

 [XXXX-XXXX-XXXX-XXXX](#) ·  [SharikHR](#)

Department of · Funded by Grant XXXXXXXX

- **None**

 [XXXX-XXXX-XXXX-XXXX](#)

Department of; Department of

## Abstract

---

Open collaborative writing with Manubot [1](#)

## Introduction

---

PDAC belongs to the top five of cancer-related deaths in the world and the poor prognosis is primarily due to its advanced stage at diagnosis, the progress in its treatment remains too slow as a consequence of the complex physiopathology of this tumor characterized by a heterogeneous cellular composition and the accumulation of a very dense fibrotic tissue [???]: Due to that pancreatic cancer is a heterogeneous disease, is often modelled using established cell lines in the laboratory.

In recent years, three-dimensional (3D) culture systems have gained increasing recognition as an effective tool for biological research. One widely used 3D culturing technique is the application of multicellular spheroids (MCS). Cells cultured in 3D more closely mimic the physiological environment compared to conventional monolayer culture systems. Spheroids are three-dimensional spherical cellular aggregates with high cell-density, that more closely simulate conditions existing in solid tumors where hypoxia and alterations related to intracellular metabolism occur due to poor availability of nutrients from blood vessels [2](#).

## Pancreatic tumor microenvironment

---

In Pancreatic ductal adenocarcinoma (PDA), the major components of the tumor microenvironment are a complex population of fibroblasts forming the bulk of the stroma, vasculature, inflammatory and immune cells [3](#). PDA is associated with evolving alterations in the tumor microenvironment, including increasing fibrosis and extracellular matrix deposition (desmoplasia). Increasing desmoplasia accompanies progressive disease and creates intratumoral pressure that compresses the vasculature, resulting in limited blood flow to the tumor and consequent hypoxia and low nutrient delivery [4](#).

Interactions between the neoplastic and non-neoplastic cells and cellular matrix have been proposed to stimulate the extensive desmoplastic reaction. Stroma production is promoted by the activation of multiple cell signalling pathways [[5](#),[6](#)] these signalling cascades lead to secretion of structural matrix components including proteoglycans, collagen and fibronectin and the activation of catalytic enzymes such as proteinases.

Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases, they are involved in degradation of the extracellular matrix. MMPs support tissue remodeling and stimulate neovascularization and inflammatory response, both in physiological and in pathological conditions, for example, in tumors [7](#).

MMP-2, MMP-7, and MMP-9 expressions correlate with various morphological features of the PDAC tumor such as inflammation, necrosis, and formation of the new blood vessels [8](#) and their tissue inhibitors (TIMPs) TIMPs 1–3 compared with normal pancreas [9](#).

Pancreatic stellate cells (PSC) are responsible for desmoplastic generation [10](#). The activation of PSC from a quiescent to an activated state is an intercellular stimuli from tumor-stromal interactions. PCS can be transform into myofibroblast-like cells, which express  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA) as cancer-associated fibroblasts (CAF) [[11](#),[12](#)].

CAF are important components of tumor stroma and affect cancer growth, survival, metastasis, angiogenesis and resistance to chemotherapy or radiotherapy through various cytokines [13](#), and contribute to a diminished immune function [14](#).

## 2D and 3D cell culture

---

Cell culture is a widely used *in vitro* tool to improve the understanding of cell biology, cellular mechanisms, tissue morphology, drug action, protein production and the development of tissue engineering [15](#).

Three-dimensional (3D) cell culture is well documented to regain intrinsic metabolic properties and to better mimic the *in vivo* situation than two-dimensional (2D) cell culture [16](#).

A comparative molecular analyses from 2D and 3D *in vitro* conditions revealed that cancer cell phenotype is highly influenced by its microenvironment. Through the examination of cancer cell transcriptional behavior, cellular processes were closely linked to functions promoted by different culturing conditions. Cancer cells grown in monolayers favored rapid proliferation, and this behavior was corroborated by up-regulation of cell cycle progression genes in addition to metabolic processes that synthesize DNA, RNA, and proteins. Unlike cancer cells cultured in monolayers, cancer cells in 3D down-regulate proliferative processes, while up-regulating genes involved in ECM organization and cell adhesion [17](#).

When performing 3D cell culture experiments, the cell environment can be manipulated to mimic that of a cell *in vivo* and provide more accurate data about cell-to-cell interactions, tumor characteristics, drug discovery, metabolic profiling, stem cell research, and other types of diseases [18](#).

Alterations in cancer cell behavior under different growth conditions underlie the importance of defining culturing conditions that preserve endogenous tumor behavior. While monolayer cultures promote the most non-native behavior of cancer cells, this model still maintains value due to its ease and scalability towards applications targeting cancer-growth driving pathways. However, analysis and discovery of potential therapeutics targeting stromal interactions, ECM development, or cell signaling may yield erroneous results in this system. The 3D culturing and inclusion of stromal cell types do show increased similarity to *in vivo* cancer behavior. However, improvements upon *ex vivo* culture conditions that allow all stromal components to persist will greatly enhance our ability to conduct pre-clinical screens that may more closely recapitulate the biological responses of patients [17](#).

## The structure of multicellular spheroids.

---

MCSs are cell aggregates with complex cell-to-cell adhesions and cell-to-matrix interactions, which results in gradient generation for nutrients, gases, growth factors and signal factors [19](#).

It has been reported that MCS formation involves three critical steps. In the first stage, dispersed single-cells are drawn closer to form aggregations where ECM fibers act as a long-chain linker through the binding of integrins. This is followed by a period in which cell aggregates pause in compaction, because of the accumulation of sufficient amounts of E-cadherins. Finally, in the third stage, cells are compacted into solid aggregates to form MCSs due to the homophilic cadherin-cadherin binding [20](#).

The formation of MCS process initiates with an spontaneous self-assembly and cell-cell interactions. These interactions are regulated by adhesions proteins such as E-cadherin,  $\alpha$ -catenin and P-cadherin [\[21,22\]](#) Cell-to-matrix interactions is the base for cell building and is influenced by integrins mainly by  $\beta 1$ -integrin. The integration of integrin-ECM facilitates the cell aggregation process that connect a cell with its environment in the context of spheroid formation [\[???,20,20\]](#).

The cytoskeleton also plays an important role in spheroid formation. During spheroid formation the actin filaments undergo significant changes and the expanded microfilaments as stress fibres become along the cell periphery. In this step, the cytoskeleton is a force generation structure performing a continuous pre-stressed lattice that keeps structural stability [23; [???](200103)48:3<175::AID-CM1007>3.0.CO;2-2].

The internal structure of spheroids comprises different cell layers. MCS include hypoxic, proliferative apoptotic/necrotic areas as a consequence of oxygen and nutrient gradients [???]. Small micro-spheroids of <200µm diameter mostly include proliferating and normoxic cells, mimic three-dimensional cell-cell and cell-matrix interactions but they are inappropriate to reflect pathophysiological conditions with hypoxic areas in the spheroid center or to mimic proliferation gradients 24. However, spheroids with diameters of approximately 200-300µm results in a typical zonation, with proliferative zones at the surface co-existing with normoxic quiescent zones in the middle and hypoxic zones in the core 25. Finally, MCS > 500 µm diameter the formation of necrotic areas is observed as described in microregion of tumor *in vivo* 26.

Due to these characteristics, MCS mimic the first avascular stages of tumor formation, and exhibit important tumor aggressiveness features such as enhanced multicellular resistance, migration, and invasion, as well as an enhanced clonogenic capacity 27.

## Methods for the generation of MCS

---

Many 3D culture techniques are available for the generation of spheroids. These methods prevent cell attachment to the culture substratum, thereby increasing interactions with neighboring cells and extracellular matrix.

Nowadays, there are four major techniques for spheroids formation by avoiding the cells adhesion to the surfaces and favoring the cell-cell interactions and cells self-aggregation.

### The agitation-based approaches.

Agitation-based approaches to culture cells as spheroids include gyratory rotation techniques, such as gyratory shakers, rotary culture systems and stirred suspension culture systems 28.

The general principle behind these methods is that a cell suspension is placed into a container and the suspension is kept in motion, that is, either it is gently stirred or the container is rotated. The continuous motion of the suspended cells allows that cells do not adhere to the containers walls, but instead form cell-cell interactions 29.

Furthermore, batches of 3D spheroids formed in spinner flasks are typically of a broad range of sizes and so would require subsequent manual selection to obtain a group of similarly sized 3D spheroids for analysis 30. Rotatory culture system function by similar means as the spinner flask but, instead of using a stirring bar to keep cell suspensions in motion, the culture container itself is rotated, enables large-scale production and allows for long-term culture of 3D spheroids [29].

### The hanging drop technique.

The hanging drop technique relies on the formation of spheroids in small drops of culture medium, which are suspended on a glass coverslip. This is a simple method in which cells are placed in hanging drop culture and incubated under physiological conditions until they form 3D spheroids. The method requires no specialized equipment and can be adapted to include addition of any biological agent in very small quantities, can also be used to co-culture two (or more) different cell populations so as to

elucidate the role of cell-cell or cell-ECM interactions in specifying spatial relationships between cells [32](#). The hanging drop method generated well-rounded MCTS and represents an attractive alternative for spheroids production, because it is mild, can be applied to a wide variety of cell lines, and can produce spheroids of a homogeneous size without the need for sieving or manual selection [33](#), for some cell lines spheroid formation occurred within 72 hours [34](#). This method has also been applied to the co-cultivation of mixed cell populations, including the co-cultivation of endothelial cells and tumor cells as a model of early tumor angiogenesis [35](#). Although they have disadvantage as producing variable size spheroids, low throughput, hard to handle, long-term culture, they provide an efficient way to obtain biological insights that are often lost in 2D platforms [36](#).

### **Liquid overlay technique.**

Liquid overlay technique includes the formation of spheroids by seeding the cells on top of non-adhesive surfaces, is one of the simplest and less expensive approaches for spheroid formation forming spheroids without size or shape limitation [37](#). In short, wells are coated with a non-adherence layer such as agarose or poly-HEMA before adding cell suspension. Besides providing a non-adhesive surface, the agarose-coated wells or ultra-low attachment plates also increase cell-to-cell contact as cells collect on their concave bottoms [38](#).

The rate of yield for this technique is between 60% and 100% for monoculture spheroids and 100% for co-culture spheroids. The size of the spheroids can be adjusted easily and precisely by varying the number of seeded cells organized in one spheroid. The formation of co-culture spheroids consisting of three different cell types is possible with this method [39](#).

### **Microfluidics.**

Microfluidics has been recently investigated for spheroids formation. Usually, microfluidics are chips composed of microchannels and microchambers, where cells suspended in media circulate and accumulate in the chambers forming the spheroids [41](#). Microfluidics, as an emerging tool to control the flow within micrometer-scale channels, is capable of manipulating the parameters dynamically and spatially, thus creating unique environments to meet the requirements for MCS formation. In addition, microfluidics can reduce the shear stress in order to minimize cell damage, and micrometer-scale chambers inside the microfluidics can be manipulated to control the size of MCS [\[??\]](#):

The microfluidic devices are designed to enhance the spheroid formation process by: providing size control; allowing rapid aggregation; requiring minimal interaction between the user and the device, and; replacing manual handling with engineered and automated procedures. These important capabilities have been achieved with the aid of transparent and biocompatible materials [41](#).

The emergence of “tumor-on-chip” technology provides an alternative to study multicellular and tumor microenvironment interactions *in vitro*. These chip devices have been extensively used to study tumor–stroma interactions, tumor-associated angiogenesis, and, recently, tumor–immune interactions [42](#).

Microfluidic channels are used to promote the formation of cellular aggregates. This method opens possibilities for the continuous production of highly controlled aggregates. However, this method currently requires a technological capacity [43](#).

### **MCS and PDA**

---

Due to that pancreatic cancer is a heterogeneous disease researchers have been developed models capable to reproduce *in vitro* the heterogeneous tumor microenvironment.

Several methods to create 3D tumors *in vitro* have been proposed, with hanging drop technique being the simplest and most frequently used. However, in many cell lines this method has failed to form the desired 3D tumor structures.

A modified hanging drop method for 3D spheroid formation facilitated with methylcellulose yield a straightforward production of spheroids in PDAC cells and form well-rounded spheroids after 5 days in hanging drops. These spheroids have high tolerance to mechanical force, thus enabling standard manipulations and display some hallmarks of solid tumors, such as hypoxic zone, proliferating cells, and apoptotic regions [44](#).

3D pancreatic cancer spheroids, based on pancreatic cancer cells and fibroblast co-culture demonstrate innate desmoplastic properties and stay poorly permeable with relevant diffusion barrier function. Spheroid-based xenografts produced different extracellular matrix (ECM) components with uniformity in terms of ECM architecture recapitulating clinical PDAC tumors. Moreover, establishment of tumors by transplantation of spheroids demonstrate higher expression of pro-fibrotic and pro-survival PDAC hallmarks [45](#).

In the last years the characterization of a novel 3D tumor model has been in full swing. A triple co-culture of pancreatic cancer cells (PANC-1), fibroblasts (MRC-5) and endothelial cells (HUVEC) to form a heterotype multicellular tumor spheroid (MCTS). The integration of the three cell types enable the presence of a core rich in fibroblasts and fibronectin in which endothelial cells are homogeneously distributed [46](#).

A microchannel model allow to develop a 3D pancreatic tumor *in vitro* by co-culturing pancreatic tumor spheroids with PSC in a collagen matrix. Under these conditions spheroids and PSCs are mutually activated when co-cultured. Under co-culture condition, tumor spheroids acquire a migratory phenotype with cancer cell-cell interactions, cell-ECM interactions, and cancer cell-PSC interactions [47](#).

## References

---

### 1. Open collaborative writing with Manubot

Daniel S. Himmelstein, Vincent Rubinetti, David R. Slochower, Dongbo Hu, Venkat S. Malladi, Casey S. Greene, Anthony Gitter  
*PLOS Computational Biology* (2019-06-24) <https://doi.org/c7np>  
DOI: [10.1371/journal.pcbi.1007128](https://doi.org/10.1371/journal.pcbi.1007128) · PMID: [31233491](https://pubmed.ncbi.nlm.nih.gov/31233491/) · PMCID: [PMC6611653](https://pubmed.ncbi.nlm.nih.gov/PMC6611653/)

### 2. Cancer cell spheroids are a better screen for the photodynamic efficiency of glycosylated photosensitizers

Patrícia M. R. Pereira, Naxhije Berisha, N. V. S. Dinesh K. Bhupathiraju, Rosa Fernandes, João P. C. Tomé, Charles Michael Drain  
*PLOS ONE* (2017-05-17) <https://doi.org/f986w7>  
DOI: [10.1371/journal.pone.0177737](https://doi.org/10.1371/journal.pone.0177737) · PMID: [28545086](https://pubmed.ncbi.nlm.nih.gov/28545086/) · PMCID: [PMC5435229](https://pubmed.ncbi.nlm.nih.gov/PMC5435229/)

### 3. Stromal biology of pancreatic cancer

Gerald C. Chu, Alec C. Kimmelman, Aram F. Hezel, Ronald A. DePinho  
*Journal of Cellular Biochemistry* (2007-07-01) <https://doi.org/b8w83q>  
DOI: [10.1002/jcb.21209](https://doi.org/10.1002/jcb.21209) · PMID: [17266048](https://pubmed.ncbi.nlm.nih.gov/17266048/)

### 4. Pancreatic Cancer Metabolism: Breaking It Down to Build It Back Up

R. M. Perera, N. Bardeesy  
*Cancer Discovery* (2015-11-03) <https://doi.org/ggt22b>  
DOI: [10.1158/2159-8290.cd-15-0671](https://doi.org/10.1158/2159-8290.cd-15-0671) · PMID: [26534901](https://pubmed.ncbi.nlm.nih.gov/26534901/) · PMCID: [PMC4687899](https://pubmed.ncbi.nlm.nih.gov/PMC4687899/)

### 5. Tumor–stromal cell interaction under hypoxia increases the invasiveness of pancreatic cancer cells through the hepatocyte growth factor/c-Met pathway

Takao Ide, Yoshihiko Kitajima, Atsushi Miyoshi, Takao Ohtsuka, Mayumi Mitsuno, Kazuma Ohtaka, Yasuo Koga, Kohji Miyazaki  
*International Journal of Cancer* (2006-12-15) <https://doi.org/bcmh42>  
DOI: [10.1002/ijc.22178](https://doi.org/10.1002/ijc.22178) · PMID: [16998831](https://pubmed.ncbi.nlm.nih.gov/16998831/)

### 6. Aberrant signaling pathways in pancreatic cancer: A two compartment view

Angela L. McCleary-Wheeler, Robert McWilliams, Martin E. Fernandez-Zapico  
*Molecular Carcinogenesis* (2012-01) <https://doi.org/dhx9xm>  
DOI: [10.1002/mc.20827](https://doi.org/10.1002/mc.20827) · PMID: [22162229](https://pubmed.ncbi.nlm.nih.gov/22162229/) · PMCID: [PMC3253704](https://pubmed.ncbi.nlm.nih.gov/PMC3253704/)

### 7. ALTERED MATRIX METALLOPROTEINASE EXPRESSION ASSOCIATED WITH ONCOGENE-MEDIATED CELLULAR TRANSFORMATION AND METASTASIS FORMATION

R Baruch  
*Cell Biology International* (2001-05) <https://doi.org/fnvccv>  
DOI: [10.1006/cbir.2000.0647](https://doi.org/10.1006/cbir.2000.0647) · PMID: [11401328](https://pubmed.ncbi.nlm.nih.gov/11401328/)

### 8. Expressions of Matrix Metalloproteinases 2, 7, and 9 in Carcinogenesis of Pancreatic Ductal Adenocarcinoma

Katarzyna Jakubowska, Anna Pryczynicz, Joanna Januszewska, Iwona Sidorkiewicz, Andrzej Kemona, Andrzej Niewiński, Łukasz Lewczuk, Bogusław Kędra, Katarzyna Guzińska-Ustymowicz  
*Disease Markers* (2016) <https://doi.org/f9hdkw>  
DOI: [10.1155/2016/9895721](https://doi.org/10.1155/2016/9895721) · PMID: [27429508](https://pubmed.ncbi.nlm.nih.gov/27429508/) · PMCID: [PMC4939209](https://pubmed.ncbi.nlm.nih.gov/PMC4939209/)

### 9. Comprehensive Analysis of Matrix Metalloproteinase and Tissue Inhibitor Expression in Pancreatic Cancer: Increased Expression of Matrix Metalloproteinase-7 Predicts Poor



## Survival

L. E. Jones

*Clinical Cancer Research* (2004-04-15) <https://doi.org/c7sfqg>

DOI: [10.1158/1078-0432.ccr-1157-03](https://doi.org/10.1158/1078-0432.ccr-1157-03) · PMID: [15102692](https://pubmed.ncbi.nlm.nih.gov/15102692/)

### 10. **Conophylline suppresses pancreatic cancer desmoplasia and cancer-promoting cytokines produced by cancer-associated fibroblasts**

Norihiro Ishii, Kenichiro Araki, Takehiko Yokobori, Kei Hagiwara, Dorgormaa Gantumur, Takahiro Yamanaka, Tadashi Handa, Mariko Tsukagoshi, Takamichi Igarashi, Akira Watanabe, ... Ken Shirabe  
*Cancer Science* (2018-12-13) <https://doi.org/ggt57m>

DOI: [10.1111/cas.13847](https://doi.org/10.1111/cas.13847) · PMID: [30353606](https://pubmed.ncbi.nlm.nih.gov/30353606/) · PMCID: [PMC6317962](https://pubmed.ncbi.nlm.nih.gov/PMC6317962/)

### 11. **A Starring Role for Stellate Cells in the Pancreatic Cancer Microenvironment**

Minoti V. Apte, Jeremy S. Wilson, Aurelia Lugea, Stephen J. Pandol

*Gastroenterology* (2013-05) <https://doi.org/f2j2rr>

DOI: [10.1053/j.gastro.2012.11.037](https://doi.org/10.1053/j.gastro.2012.11.037) · PMID: [23622130](https://pubmed.ncbi.nlm.nih.gov/23622130/) · PMCID: [PMC3729446](https://pubmed.ncbi.nlm.nih.gov/PMC3729446/)

### 12. **CD10+ Pancreatic Stellate Cells Enhance the Progression of Pancreatic Cancer**

Naoki Ikenaga, Kenoki Ohuchida, Kazuhiro Mizumoto, Lin Cui, Tadashi Kayashima, Katsuya Morimatsu, Taiki Moriyama, Kohei Nakata, Hayato Fujita, Masao Tanaka

*Gastroenterology* (2010-09) <https://doi.org/cps2bn>

DOI: [10.1053/j.gastro.2010.05.084](https://doi.org/10.1053/j.gastro.2010.05.084) · PMID: [20685603](https://pubmed.ncbi.nlm.nih.gov/20685603/)

### 13. **Cancer-associated fibroblasts in pancreatic adenocarcinoma**

Boju Pan, Quan Liao, Zheyu Niu, Li Zhou, Yupei Zhao

*Future Oncology* (2015-09) <https://doi.org/f7sxbh>

DOI: [10.2217/fon.15.176](https://doi.org/10.2217/fon.15.176) · PMID: [26284509](https://pubmed.ncbi.nlm.nih.gov/26284509/)

### 14. **Human Pancreatic Carcinoma-Associated Fibroblasts Promote Expression of Co-inhibitory Markers on CD4+ and CD8+ T-Cells**

Laia Gorchs, Carlos Fernández Moro, Peter Bankhead, Katharina P. Kern, Imrul Sadeak, Qingda Meng, Elena Rangelova, Helen Kaipe

*Frontiers in Immunology* (2019-04-24) <https://doi.org/ggt57p>

DOI: [10.3389/fimmu.2019.00847](https://doi.org/10.3389/fimmu.2019.00847) · PMID: [31068935](https://pubmed.ncbi.nlm.nih.gov/31068935/) · PMCID: [PMC6491453](https://pubmed.ncbi.nlm.nih.gov/PMC6491453/)

### 15. **2D and 3D cell cultures – a comparison of different types of cancer cell cultures**

Marta Kapalczyńska, Tomasz Kolenda, Weronika Przybyła, Maria Zajączkowska, Anna Teresiak, Violetta Filas, Matthew Ibbs, Renata Bliźniak, Łukasz Łuczewski, Katarzyna Lamperska

*Archives of Medical Science* (2016) <https://doi.org/ggt3hn>

DOI: [10.5114/aoms.2016.63743](https://doi.org/10.5114/aoms.2016.63743) · PMID: [30002710](https://pubmed.ncbi.nlm.nih.gov/30002710/) · PMCID: [PMC6040128](https://pubmed.ncbi.nlm.nih.gov/PMC6040128/)

### 16. **Oncogenic human herpesvirus hijacks proline metabolism for tumorigenesis**

Un Yung Choi, Jae Jin Lee, Angela Park, Wei Zhu, Hye-Ra Lee, Youn Jung Choi, Ji-Seung Yoo, Claire Yu, Pinghui Feng, Shou-Jiang Gao, ... Jae U. Jung

*Proceedings of the National Academy of Sciences* (2020-04-07) <https://doi.org/ggt4fr>

DOI: [10.1073/pnas.1918607117](https://doi.org/10.1073/pnas.1918607117) · PMID: [32213586](https://pubmed.ncbi.nlm.nih.gov/32213586/) · PMCID: [PMC7149499](https://pubmed.ncbi.nlm.nih.gov/PMC7149499/)

### 17. **Comparative Molecular Analysis of Cancer Behavior Cultured In Vitro, In Vivo, and Ex Vivo**

Nicholas R. Hum, Aimy Sebastian, Sean F. Gilmore, Wei He, Kelly A. Martin, Aubree Hinckley, Karen R. Dubbin, Monica L. Moya, Elizabeth K. Wheeler, Matthew A. Coleman, Gabriela G. Loots

*Cancers* (2020-03-14) <https://doi.org/ggt4ft>

DOI: [10.3390/cancers12030690](https://doi.org/10.3390/cancers12030690) · PMID: [32183351](https://pubmed.ncbi.nlm.nih.gov/32183351/) · PMCID: [PMC7140030](https://pubmed.ncbi.nlm.nih.gov/PMC7140030/)



18. **Is It Time to Start Transitioning From 2D to 3D Cell Culture?**  
Caleb Jensen, Yong Teng  
*Frontiers in Molecular Biosciences* (2020-03-06) <https://doi.org/ggt4fs>  
DOI: [10.3389/fmolb.2020.00033](https://doi.org/10.3389/fmolb.2020.00033) · PMID: [32211418](https://pubmed.ncbi.nlm.nih.gov/32211418/) · PMCID: [PMC7067892](https://pubmed.ncbi.nlm.nih.gov/PMC7067892/)
19. **Advances in multicellular spheroids formation**  
X. Cui, Y. Hartanto, H. Zhang  
*Journal of The Royal Society Interface* (2017-02) <https://doi.org/ggt8pm>  
DOI: [10.1098/rsif.2016.0877](https://doi.org/10.1098/rsif.2016.0877) · PMID: [28202590](https://pubmed.ncbi.nlm.nih.gov/28202590/) · PMCID: [PMC5332573](https://pubmed.ncbi.nlm.nih.gov/PMC5332573/)
20. **Dynamic analysis of hepatoma spheroid formation: roles of E-cadherin and  $\beta$ 1-integrin**  
Ruei-Zeng Lin, Li-Fang Chou, Chi-Chen Michael Chien, Hwan-You Chang  
*Cell and Tissue Research* (2006-02-18) <https://doi.org/dx2zbb>  
DOI: [10.1007/s00441-005-0148-2](https://doi.org/10.1007/s00441-005-0148-2) · PMID: [16489443](https://pubmed.ncbi.nlm.nih.gov/16489443/)
21. **E-cadherin, actin, microtubules and FAK dominate different spheroid formation phases and important elements of tissue integrity**  
I. Smyrek, B. Mathew, S. C. Fischer, S. M. Lissek, S. Becker, E. H. K. Stelzer  
*Biology Open* (2019-01-15) <https://doi.org/ggt8pn>  
DOI: [10.1242/bio.037051](https://doi.org/10.1242/bio.037051) · PMID: [30578251](https://pubmed.ncbi.nlm.nih.gov/30578251/) · PMCID: [PMC6361217](https://pubmed.ncbi.nlm.nih.gov/PMC6361217/)
22. **Diversity of cell-mediated adhesions in breast cancer spheroids**  
Andrea Ivascu, Manfred Kubbies  
*International Journal of Oncology* (2007-12-01) <https://doi.org/gf8czq>  
DOI: [10.3892/ijo.31.6.1403](https://doi.org/10.3892/ijo.31.6.1403)
23. **Rapid Enhancement of Cellular Spheroid Assembly by Acoustically Driven Microcentrifugation**  
Layla Alhasan, Aisha Qi, Aswan Al-Abboodi, Amgad Rezk, Peggy P. Y. Chan, Ciprian Iliescu, Leslie Y. Yeo  
*ACS Biomaterials Science & Engineering* (2016-05-18) <https://doi.org/ggt8pj>  
DOI: [10.1021/acsbiomaterials.6b00144](https://doi.org/10.1021/acsbiomaterials.6b00144)
24. **Formation of size-controllable tumour spheroids using a microfluidic pillar array ( $\mu$ FPA) device**  
Wanyoung Lim, Hong-Hoa Hoang, Daeun You, Jeonghun Han, Jeong Eon Lee, Sangmin Kim, Sungsu Park  
*The Analyst* (2018) <https://doi.org/gfnsm>  
DOI: [10.1039/c8an01752b](https://doi.org/10.1039/c8an01752b) · PMID: [30379148](https://pubmed.ncbi.nlm.nih.gov/30379148/)
25. **3D tumor spheroid models for in vitro therapeutic screening: a systematic approach to enhance the biological relevance of data obtained**  
Michele Zanoni, Filippo Piccinini, Chiara Arienti, Alice Zamagni, Spartaco Santi, Rolando Polico, Alessandro Bevilacqua, Anna Tesei  
*Scientific Reports* (2016-01-11) <https://doi.org/ggt8pk>  
DOI: [10.1038/srep19103](https://doi.org/10.1038/srep19103) · PMID: [26752500](https://pubmed.ncbi.nlm.nih.gov/26752500/) · PMCID: [PMC4707510](https://pubmed.ncbi.nlm.nih.gov/PMC4707510/)
26. **Spherical Cancer Models in Tumor Biology**  
Louis-Bastien Weiswald, Dominique Bellet, Virginie Dangles-Marie  
*Neoplasia* (2015-01) <https://doi.org/f6xf5r>  
DOI: [10.1016/j.neo.2014.12.004](https://doi.org/10.1016/j.neo.2014.12.004) · PMID: [25622895](https://pubmed.ncbi.nlm.nih.gov/25622895/) · PMCID: [PMC4309685](https://pubmed.ncbi.nlm.nih.gov/PMC4309685/)

27. **Comparison of 2D- and 3D-culture models as drug-testing platforms in breast cancer**  
YOSHINORI IMAMURA, TORU MUKOHARA, YOHEI SHIMONO, YOHEI FUNAKOSHI, NAOKO CHAYAHARA, MASANORI TOYODA, NAOMI KIYOTA, SHINTARO TAKAO, SEISHI KONO, TETSUYA NAKATSURA, HIRONOBU MINAMI  
*Oncology Reports* (2015-04) <https://doi.org/ggt8pp>  
DOI: [10.3892/or.2015.3767](https://doi.org/10.3892/or.2015.3767) · PMID: [25634491](https://pubmed.ncbi.nlm.nih.gov/25634491/)
28. **Three-dimensional tissue culture models in cancer biology**  
Jong Bin Kim  
*Seminars in Cancer Biology* (2005-10) <https://doi.org/dk5d92>  
DOI: [10.1016/j.semcancer.2005.05.002](https://doi.org/10.1016/j.semcancer.2005.05.002) · PMID: [15975824](https://pubmed.ncbi.nlm.nih.gov/15975824/)
29. **Three-dimensional cell culture: the missing link in drug discovery**  
Susan Breslin, Lorraine O'Driscoll  
*Drug Discovery Today* (2013-03) <https://doi.org/ggt8tt>  
DOI: [10.1016/j.drudis.2012.10.003](https://doi.org/10.1016/j.drudis.2012.10.003) · PMID: [23073387](https://pubmed.ncbi.nlm.nih.gov/23073387/)
30. **Recent advances in three-dimensional multicellular spheroid culture for biomedical research**  
Ruei-Zhen Lin, Hwan-You Chang  
*Biotechnology Journal* (2008-10) <https://doi.org/b2zq2w>  
DOI: [10.1002/biot.200700228](https://doi.org/10.1002/biot.200700228) · PMID: [18566957](https://pubmed.ncbi.nlm.nih.gov/18566957/)
31. **Organotypic 3D cell culture models: using the rotating wall vessel to study host-pathogen interactions**  
Jennifer Barrila, Andrea L. Radtke, Aurélie Crabbé, Shameema F. Sarker, Melissa M. Herbst-Kralovetz, C. Mark Ott, Cheryl A. Nickerson  
*Nature Reviews Microbiology* (2010-10-15) <https://doi.org/bx4352>  
DOI: [10.1038/nrmicro2423](https://doi.org/10.1038/nrmicro2423) · PMID: [20948552](https://pubmed.ncbi.nlm.nih.gov/20948552/)
32. **A Simple Hanging Drop Cell Culture Protocol for Generation of 3D Spheroids**  
Ramsey Foty  
*Journal of Visualized Experiments* (2011-05-06) <https://doi.org/fdn8n9>  
DOI: [10.3791/2720](https://doi.org/10.3791/2720) · PMID: [21587162](https://pubmed.ncbi.nlm.nih.gov/21587162/) · PMCID: [PMC3197119](https://pubmed.ncbi.nlm.nih.gov/PMC3197119/)
33. **Method for generation of homogeneous multicellular tumor spheroids applicable to a wide variety of cell types**  
Jens M. Kelm, Nicholas E. Timmins, Catherine J. Brown, Martin Fussenegger, Lars K. Nielsen  
*Biotechnology and Bioengineering* (2003-07-20) <https://doi.org/drzwsj>  
DOI: [10.1002/bit.10655](https://doi.org/10.1002/bit.10655) · PMID: [12768623](https://pubmed.ncbi.nlm.nih.gov/12768623/)
34. **Is "Hanging Drop" a Useful Method to Form Spheroids of Jimt, Mcf-7, T-47d, Bt-474 That are Breast Cancer Cell Lines**  
Yilmaz O, Sakarya S  
*Single Cell Biology* (2018) <https://doi.org/ggt8tx>  
DOI: [10.4172/2168-9431.1000170](https://doi.org/10.4172/2168-9431.1000170)
35. **Hanging-drop multicellular spheroids as a model of tumour angiogenesis**  
Nicholas Timmins, Stefanie Dietmair, Lars Nielsen  
*Angiogenesis* (2004) <https://doi.org/drkw6h>  
DOI: [10.1007/s10456-004-8911-7](https://doi.org/10.1007/s10456-004-8911-7) · PMID: [15516830](https://pubmed.ncbi.nlm.nih.gov/15516830/)

36. **High-throughput 3D spheroid culture and drug testing using a 384 hanging drop array**  
Yi-Chung Tung, Amy Y. Hsiao, Steven G. Allen, Yu-suke Torisawa, Mitchell Ho, Shuichi Takayama  
*The Analyst* (2011) <https://doi.org/b8hng4>  
DOI: [10.1039/c0an00609b](https://doi.org/10.1039/c0an00609b) · PMID: [20967331](https://pubmed.ncbi.nlm.nih.gov/20967331/)
37. **Spheroid culture as a tool for creating 3D complex tissues**  
Eelco Fennema, Nicolas Rivron, Jeroen Rouwkema, Clemens van Blitterswijk, Jan de Boer  
*Trends in Biotechnology* (2013-02) <https://doi.org/f4j3r8>  
DOI: [10.1016/j.tibtech.2012.12.003](https://doi.org/10.1016/j.tibtech.2012.12.003) · PMID: [23336996](https://pubmed.ncbi.nlm.nih.gov/23336996/)
38. **Spheroid-based drug screen: considerations and practical approach**  
Juergen Friedrich, Claudia Seidel, Reinhard Ebner, Leoni A Kunz-Schughart  
*Nature Protocols* (2009-02-12) <https://doi.org/dg3kwv>  
DOI: [10.1038/nprot.2008.226](https://doi.org/10.1038/nprot.2008.226) · PMID: [19214182](https://pubmed.ncbi.nlm.nih.gov/19214182/)
39. **The liquid overlay technique is the key to formation of co-culture spheroids consisting of primary osteoblasts, fibroblasts and endothelial cells**  
Wolfgang Metzger, Daniela Sossong, Annick Bächle, Norbert Pütz, Gunther Wennemuth, Tim Pohlemann, Martin Oberringer  
*Cytotherapy* (2011-09) <https://doi.org/bqbf5p>  
DOI: [10.3109/14653249.2011.583233](https://doi.org/10.3109/14653249.2011.583233) · PMID: [21619419](https://pubmed.ncbi.nlm.nih.gov/21619419/)
40. **Optimization of liquid overlay technique to formulate heterogenic 3D co-cultures models**  
Elisabete C. Costa, Vítor M. Gaspar, Paula Coutinho, Ilídio J. Correia  
*Biotechnology and Bioengineering* (2014-08) <https://doi.org/f6f883>  
DOI: [10.1002/bit.25210](https://doi.org/10.1002/bit.25210) · PMID: [24615162](https://pubmed.ncbi.nlm.nih.gov/24615162/)
41. **Spheroids-on-a-chip: Recent advances and design considerations in microfluidic platforms for spheroid formation and culture**  
Khashayar Moshksayan, Navid Kashaninejad, Majid Ebrahimi Warkiani, John G. Lock, Hajar Moghadas, Bahar Firoozabadi, Mohammad Said Saidi, Nam-Trung Nguyen  
*Sensors and Actuators B: Chemical* (2018-06) <https://doi.org/ggt8tv>  
DOI: [10.1016/j.snb.2018.01.223](https://doi.org/10.1016/j.snb.2018.01.223)
42. **An Engineered Tumor-on-a-Chip Device with Breast Cancer-Immune Cell Interactions for Assessing T-cell Recruitment**  
Aereas Aung, Vardhman Kumar, Jomkuan Theprungsirikul, Shruti K. Davey, Shyni Varghese  
*Cancer Research* (2020-01-15) <https://doi.org/ggt8tw>  
DOI: [10.1158/0008-5472.can-19-0342](https://doi.org/10.1158/0008-5472.can-19-0342) · PMID: [31744818](https://pubmed.ncbi.nlm.nih.gov/31744818/)
43. **Efficient formation of uniform-sized embryoid bodies using a compartmentalized microchannel device**  
Yu-suke Torisawa, Bor-han Chueh, Dongeun Huh, Poornapriya Ramamurthy, Therese M. Roth, Kate F. Barald, Shuichi Takayama  
*Lab on a Chip* (2007) <https://doi.org/dq2pd7>  
DOI: [10.1039/b618439a](https://doi.org/10.1039/b618439a) · PMID: [17538720](https://pubmed.ncbi.nlm.nih.gov/17538720/)
44. **Generation of Homogenous Three-Dimensional Pancreatic Cancer Cell Spheroids Using an Improved Hanging Drop Technique**  
Matthew J. Ware, Kevin Colbert, Vazrik Keshishian, Jason Ho, Stuart J. Corr, Steven A. Curley, Biana Godin  
*Tissue Engineering Part C: Methods* (2016-04) <https://doi.org/f8f74k>  
DOI: [10.1089/ten.tec.2015.0280](https://doi.org/10.1089/ten.tec.2015.0280) · PMID: [26830354](https://pubmed.ncbi.nlm.nih.gov/26830354/) · PMCID: [PMC4827286](https://pubmed.ncbi.nlm.nih.gov/PMC4827286/)

45. **Subcutaneous Inoculation of 3D Pancreatic Cancer Spheroids Results in Development of Reproducible Stroma-Rich Tumors**  
Mikhail Durymanov, Christian Kroll, Anastasia Permyakova, Elizabeth O'Neill, Raed Sulaiman, Michael Person, Joshua Reineke  
*Translational Oncology* (2019-01) <https://doi.org/ggt6j8>  
DOI: [10.1016/j.tranon.2018.10.003](https://doi.org/10.1016/j.tranon.2018.10.003) · PMID: [30554606](https://pubmed.ncbi.nlm.nih.gov/30554606/) · PMCID: [PMC6295361](https://pubmed.ncbi.nlm.nih.gov/PMC6295361/)
46. **Multicellular spheroid based on a triple co-culture: A novel 3D model to mimic pancreatic tumor complexity**  
Gianpiero Lazzari, Valérie Nicolas, Michiya Matsusaki, Mitsuru Akashi, Patrick Couvreur, Simona Mura  
*Acta Biomaterialia* (2018-09) <https://doi.org/ggt6j7>  
DOI: [10.1016/j.actbio.2018.08.008](https://doi.org/10.1016/j.actbio.2018.08.008) · PMID: [30099198](https://pubmed.ncbi.nlm.nih.gov/30099198/)
47. **Microfluidic co-culture of pancreatic tumor spheroids with stellate cells as a novel 3D model for investigation of stroma-mediated cell motility and drug resistance**  
Ji-Hyun Lee, Seul-Ki Kim, Iftikhar Ali Khawar, Su-Yeong Jeong, Seok Chung, Hyo-Jeong Kuh  
*Journal of Experimental & Clinical Cancer Research* (2018-01-12) <https://doi.org/ggt6j9>  
DOI: [10.1186/s13046-017-0654-6](https://doi.org/10.1186/s13046-017-0654-6) · PMID: [29329547](https://pubmed.ncbi.nlm.nih.gov/29329547/) · PMCID: [PMC5767067](https://pubmed.ncbi.nlm.nih.gov/PMC5767067/)