Technology Networks

APPLICATIONS OF 3D CULTURE

Cell culture has played an important role in advancing biological and biomedical research since the technique was first developed over 100 years ago.

Traditional two-dimensional (2D) formats are progressively being replaced with increasingly advanced three-dimensional (3D) cultures that more closely mimic the *in vivo* environment and support a wider range of applications.

This infographic will highlight the advantages that 3D cell culture offers and explore some of the applications 3D models are benefitting.

WHY MOVE TO 3D?

2D CULTURES

2D cultures of monolayers of cells grown on flat, planar surfaces are convenient, cost-effective and easy to grow, but have several limitations:

- Show limited cell–cell interaction
- Demonstrate disrupted cellular organization
- Often transformed or genetically immortalized
- Poorly reproduce the cellular microenvironment
- Less effective at predicting *in vivo* toxicity and as preclinical models for drug screening

3D CULTURES

3D cultures offer numerous advantages, helping to bridge the gap between animal models and 2D cultures.

- Structurally more representative
- Greater physiological relevance
- Better reproduce the cellular microenvironment
- Avoid ethical implications of using animal models
- Able to model human-specific molecular and/or cellular pathomechanisms
- Usually easier to generate than a humanized mouse model
- Enable higher-throughput screening than animal models

3D CULTURE APPROACHES

There are a variety of 3D culture techniques available, including:

HANGING DROP
Cell suspensions are dispensed into access holes on the top of the plates, where they aggregate to form spheroids.

LOW ADHESION MICROPLATES
Cells are prevented from attaching to the plates by special coatings on the surface of the plates, allowing spheroids to form.

MAGNETIC LEVITATION
An externally applied magnetic field is used to move cells preloaded with magnetic nanoparticles toward the air/liquid interface.

BIOREACTOR-BASED
High-speed stirring and movement of media prevent attachment of cells and promote transport of nutrients and removal of waste.

HYDROGELS
Crosslinked polymer chains that possess high water content and a soft tissue-like stiffness.

SOLID SCAFFOLDS
Cells are seeded into a rigid scaffold made from materials such as polystyrene or polyamide.

MICROFLUIDICS
Cells are seeded into a chamber. Adjustable fluid flow and mechanical forces mimic dynamic physiological processes. Multi-organ setups can study interorgan crosstalk.

WHAT APPLICATIONS IS 3D CULTURE BENEFITTING?

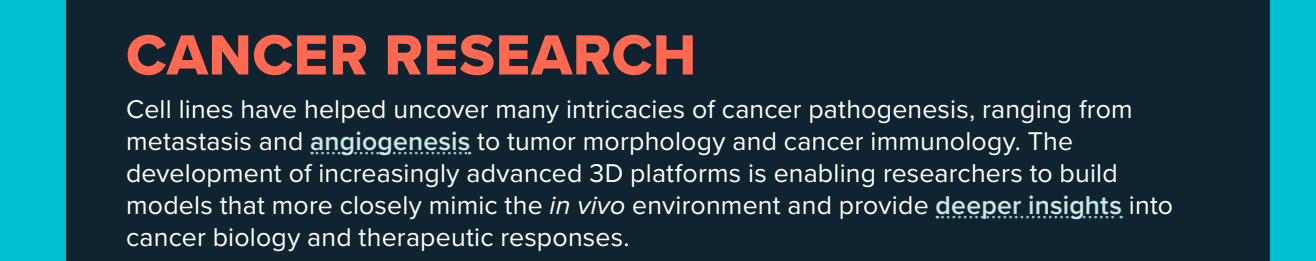
DISEASE MODELING

3D models are an attractive tool to study diseases ranging from cancer to COVID-19.

INFECTIOUS DISEASES

Although 2D and animal models have enabled important insights to be gained into a range of infectious diseases, they have been unable to reproduce certain molecular and cellular hallmarks of natural infection and the human disease process.

3D models could offer solutions to overcome these challenges, providing a better model of the host, and its interplay with microorganisms.



COVID-19 – Brain, lung, liver and gastrointestinal organoids, among others, have been used to gain understanding of SARS-CoV-2 infection.

Zika virus – Brain organoids were used to study Zika virus-induced microcephaly.

Salmonella – Enteroids and induced intestinal organoids have been used to model bacteria–host interactions of *Salmonella* infection.

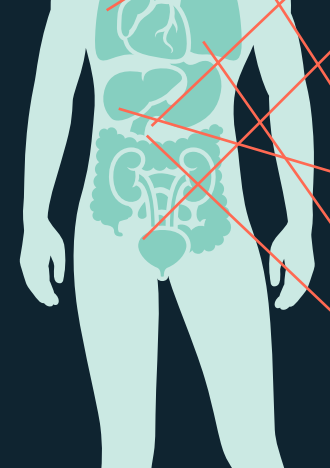
Helicobacter pylori – Gastric organoids have helped study the pathogenesis of *H. pylori*.

***Pneumocystis* species** – The potential of lung organoids to further the study of *Pneumocystis* species was demonstrated.

Malaria – Organoids can provide greater insights into *Plasmodium* infection.

CANCER RESEARCH

Cell lines have helped uncover many intricacies of cancer pathogenesis, ranging from metastasis and angiogenesis to tumor morphology and cancer immunology. The development of increasingly advanced 3D platforms is enabling researchers to build models that more closely mimic the *in vivo* environment and provide deeper insights into cancer biology and therapeutic responses.



Breast cancer – Breast cancer organoids were used to highlight metabolic changes that fuel tumor recurrence.

Pancreatic cancer – Pancreatic organoids were used to identify chemotherapeutic susceptibilities.

Prostate cancer – A 3D cell culture model was used to investigate pathogenesis of prostate cancer.

Glioma – A 3D model was used to study glioma stemness.

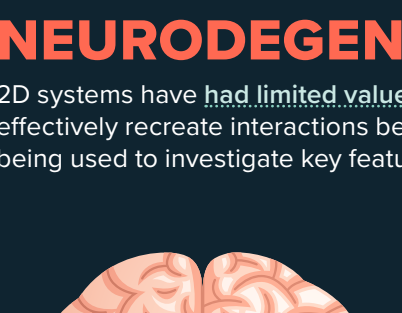
Liver cancer – Liver organoids have revealed intratumor and interpatient heterogeneity and its impact on drug response.

Lung cancer –Tumor cell line 3D spheroid models were used to study lung tumor biology.

Patient-derived organoids could help to drive forward the field of precision oncology, providing information for oncologists to truly individualize treatments.

NEURODEGENERATIVE DISEASES

2D systems have had limited value as neurodegenerative disease models, as they do not effectively recreate interactions between cells. Organ-like model systems are increasingly being used to investigate key features of several neurodegenerative diseases.



Alzheimer's disease – Brain organoids can recapitulate key features of Alzheimer's disease pathophysiology.

Parkinson's disease – Midbrain-like organoids were developed to model Parkinson's disease.

Multiple sclerosis – Organotypic brain cultures were used to study demyelination and remyelination.

Huntingdon's disease – An on-a-chip approach was used to reconstitute a Huntingdon's disease corticostriatal network.

Amyotrophic lateral sclerosis (ALS) - ALS-on-a-chip technology was developed to help to elucidate the pathogenesis of ALS.

DRUG DISCOVERY AND DEVELOPMENT

The overall failure rate in drug development is estimated to be over 96%.

Drugs that fail to make it through the drug development pipeline result in a huge waste of time, money and resources. Preclinical experiments using 2D cell culture systems are often poorly predictive of human efficacy and cells grown in 3D culture have been shown to be more resistant to drugs than those grown in 2D.

The use of improved, more physiologically relevant 3D cultures could help to better predict drug candidate efficacy and safety and identify unsuitable compounds earlier on in the drug discovery process.

TARGET IDENTIFICATION

A high-throughput screening approach using a 3D spheroid-based culture format was used to identify drugs that target KRAS.

Oncospheres generated from a human glioblastoma were shown to both accurately mimic the genetic and phenotypic characteristics of the original tumor and be amenable to high-throughput drug screening.

DRUG EFFICACY

3D colorectal carcinoma co-culture systems were shown to be robust, reproducible and well suited for drug efficacy studies.

Colorectal cancer organoids enabled the investigation of CAR NK therapy efficacy.

DRUG TOXICITY

Human organoids show great potential to study the metabolism, transport and toxicity of drugs prior to commencing human clinical trials, helping to predict drug responses earlier:

Drug-induced gastrointestinal toxicity
Primary human ileal organoids were used to predict drug-induced diarrhea of 31 drugs.

Drug-induced hepatotoxicity
3D hepatic organoids demonstrated increased sensitivity to drug-induced phospholipidosis than conventional 2D cultured cells.

Drug-induced cardiotoxicity
3D cardiac microtissues were used to assess 15 FDA-approved structural cardiotoxins and 14 FDA-approved non-structural cardiotoxins.

Drug-induced kidney injury
Induced pluripotent stem cell-derived human kidney organoids were used to model cisplatin-induced injury.

PRECISION MEDICINE

Patient-derived organoids could be used to directly test the drug sensitivity of an individual patient's tumor, helping to improve response and reduce side effects.

Personalized treatment of other diseases, such as cystic fibrosis, could also be guided by organoids created from patient-derived cells.

REGENERATIVE MEDICINE

The potential of 3D culture to revolutionize regenerative medicine is a rapidly growing area of interest.

Cells grown in 3D models are being investigated as treatments for a range of diseases and conditions:

- Post–myocardial infarction repair
- Bone repair
- Endogenous kidney regeneration
- Repairing ulcers in inflammatory bowel disease
- Retinal degeneration

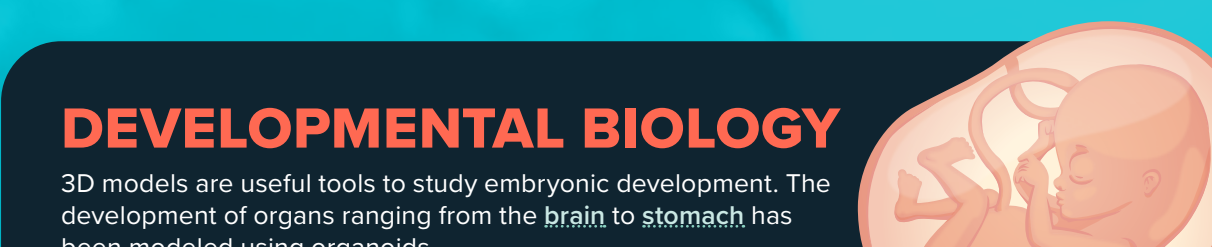
DEVELOPMENTAL BIOLOGY

3D models are useful tools to study embryonic development. The development of organs ranging from the brain to stomach has been modeled using organoids.

CULTURED MEAT

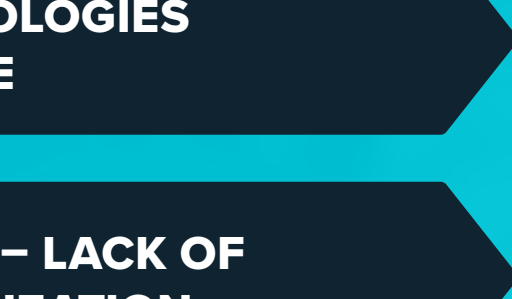
Cultured meat is seen by many as a more sustainable way to meet the increasing global demand for meat, minimizing environmental and ethical issues associated with traditional livestock farming.

A variety of 3D culture methods can be applied to construct cultured meat. Scaffolding could play a crucial role in the scalability of cultured meat. Animal-free materials are desirable for this purpose, to reduce variability, and address environmental and ethical concerns.



COSMETICS TESTING

Advanced 3D skin models are increasingly being sought as alternatives to animal models to evaluate epidermal and dermal responses to cosmetics.



CHALLENGES

Despite the advantages that 3D culture can offer, there are still some limitations associated with its use:

01 COST – 3D TECHNOLOGIES CAN BE EXPENSIVE

02 REPRODUCIBILITY – LACK OF MODEL STANDARDIZATION

03 DATA ANALYSIS CHALLENGES

04 COMPLEXITY AND HETEROGENEITY

05 SCALABILITY – THROUGHPUT IS OFTEN LOW