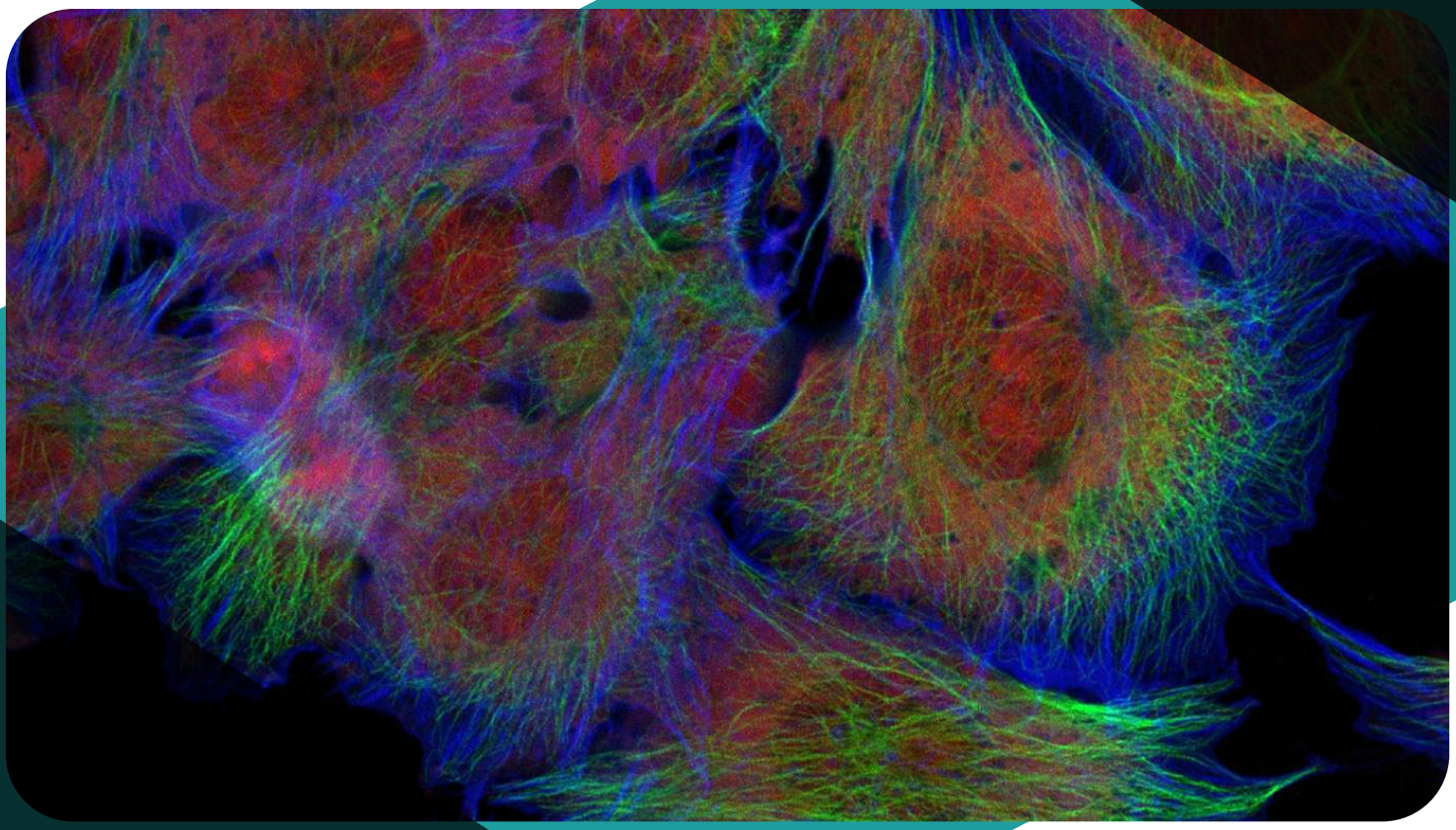
BioTechniques Spotlight



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eBook: Advanced cell models

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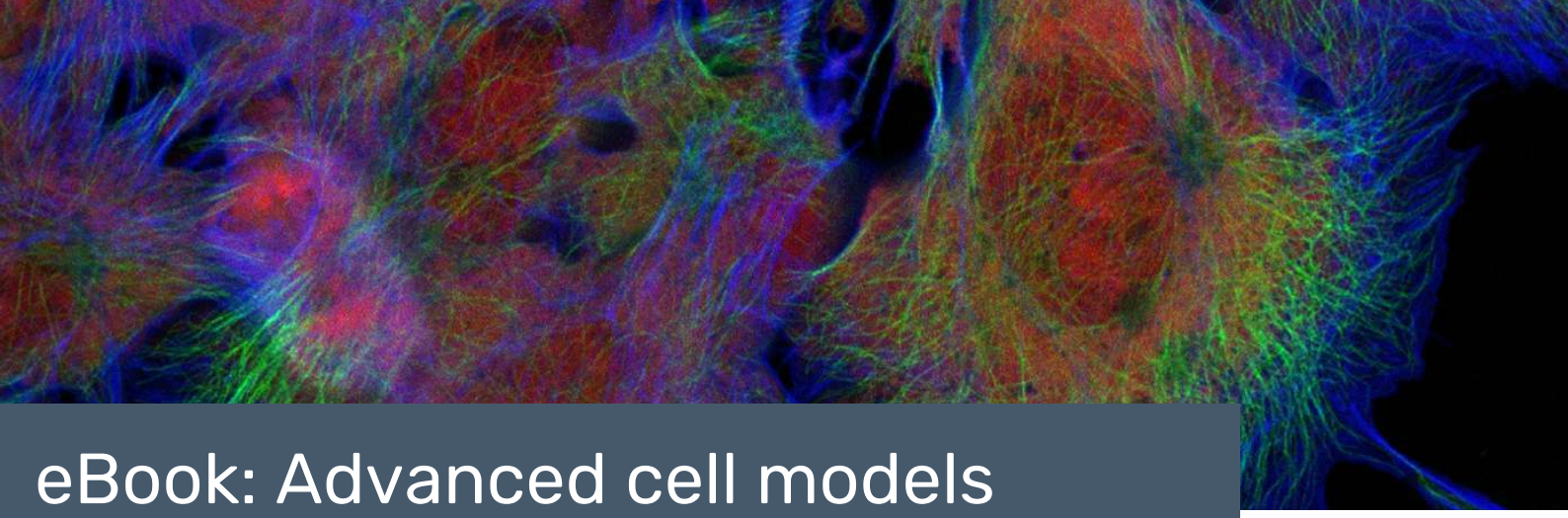

eBook: Advanced cell models

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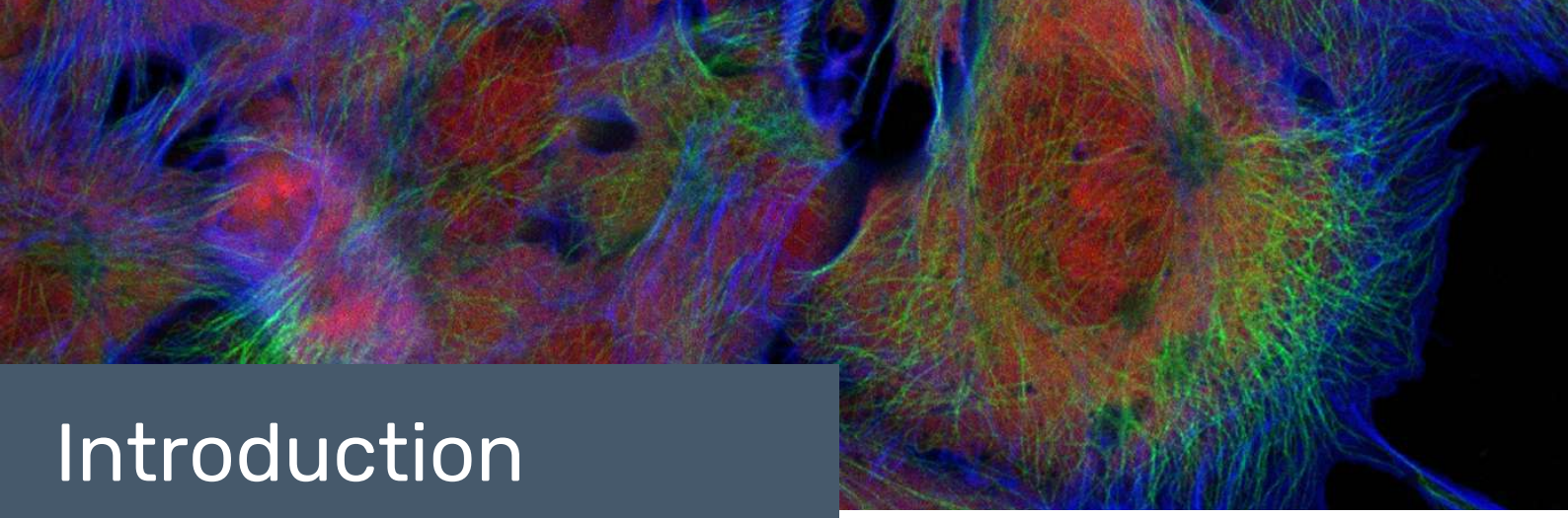

Introduction

Advanced cell cultures represent a new window through which researchers can observe the developmental biology of organs, the underlying pathogenesis and progression of diseases and the impact of therapeutics on both diseased and healthy tissues.

The increased parity to *in vivo* conditions provided by 3D cell cultures imbues these insights with a greater, more actionable value than 2D cultures, while organ-on-a-chip systems allow for refined control of experimental conditions.

However, with this increased value comes great challenges. Imaging and observation of the full cross-section of 3D cell cultures can prove difficult and scaling experiments with organ-on-a-chip systems can prove challenging. Combined with high expenses and issues of reproducibility, these models can, at times, seem imposing.

In this eBook, you will find updates and expert insights into the latest in the realm of advanced cell models, their applications in disease research and drug discovery and their analysis. Explore how these models have developed in recent years to become increasingly representative of the *in vivo* environment. Discoer how combinations of these pioneering models can be used to answer complex biological questions and hear from a legend of the field on the history and development of these models.

We hope you enjoy this ebook!

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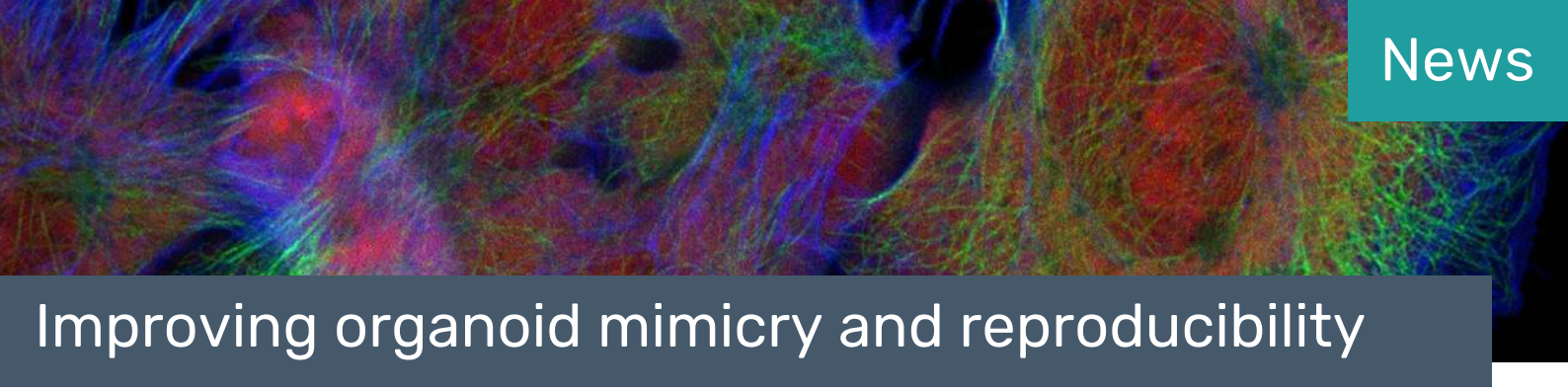


Improving organoid mimicry and reproducibility

A new synthetic gel enables the generation of representative, reliable pancreatic organoid models of healthy and cancerous tissue and many other tissue types.

A recent collaboration between the Cancer Research UK Manchester Institute and MIT (MA, USA), led by Claus Jorgensen and Linda Griffith, respectively, has implemented a new protocol to produce pancreatic organoids. The method relies on a new gel that delivers a more reproducible and accurate model of both healthy and cancerous pancreatic tissue.

A better gel for better organoids

A recurrent issue in the development and implementation of organoid technologies is the variability of their composition due, in part, to the tissue-derived gels that are frequently used as scaffolds for their growth. According to Griffith, the most commonly used of these gels can vary from batch to batch and can also limit the number of cell types that can grow in the 3D culture.

To address these limitations, Griffith set out almost a decade ago to produce a synthetic gel that could support multiple cell types. After extensive research of the biophysical and biochemical properties of the extracellular matrix, which the gel acts almost as a stand-in for, Griffith incorporated key components of this matrix into a polyethylene glycol gel. Peptide ligands that interact with integrins on the surface of cells enabled cells to bind to the gel, while synthetic peptides derived from fibronectin and collagen permitted the growth of different epithelial tissues, complete with supporting stromal cells.

Capturing cancer

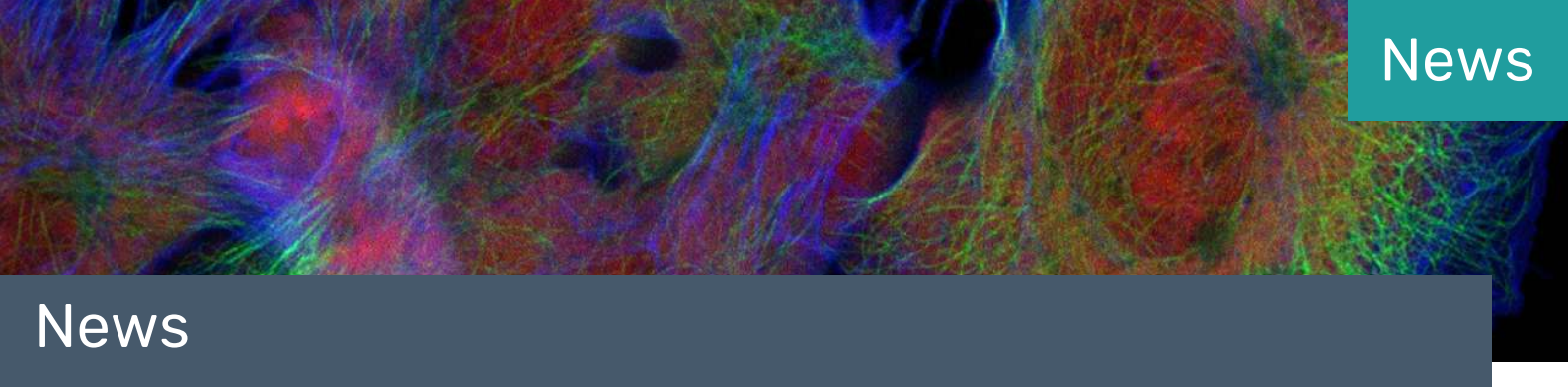
With her new gel firmly established, Griffith teamed up with Jorgensen to see if it could help resolve a common issue in pancreatic cancer modeling. When pancreatic tumor cells are excised from their native tissue, they can lose their cancerous characteristics, leading to inaccurate recreations of these tumors.

To see if her gel would successfully generate accurate pancreatic organoids, Griffith provided Jorgensen with a protocol for the gel's production. Once Jorgensen had all the reagents required and began to apply the protocol, "…it just worked!" He exclaimed, noting how simple it was to derive pancreatic organoids from both healthy and cancerous cell lines. "I think that speaks volumes of how robust the system is and how easy it is to implement in the lab."

Comparing these organoids to cancerous pancreatic tissue from mouse models, the team noted several similarities in the integrins expressed. What's more, cells commonly found in the tumor microenvironment, including macrophages and fibroblasts, were also able to thrive within these new models.

Patient-derived pancreatic organoids

Looking at the translational capabilities of these models, the team assessed their ability to create patient-derived organoids – one of the most exciting



News

contemporary applications of organoids – and were pleased to find that they were capable of the feat. Patient-derived organoids can be used to screen a specific therapeutic regimen to assess its potential for success in treating an individual patient; this represents a significant step towards a personalized medicine approach to cancer therapeutics.

With this capability validated, Griffith intends to develop patient-derived organoid models of endometriosis – a condition characterized by the growth of the tissue of the uterine lining outside the uterus.

As the gel used to create these organoids is synthetic, it can be created reliably using a simple workflow that yields a gel of the same composition each time. This makes methods reporting much simpler and therefore, the repetition of work by other labs much easier, allowing researchers to build on these studies and dramatically increasing the value of results derived from these organoids. With reproducibility constantly at the forefront of biomedical research, particularly in a new and emerging field, technologies such as this that increasingly standardize processes are in high demand.

Investigating how UTIs recur with organoid and organ-on-a-chip technology

Two new studies utilize novel organoid and organ-on-a-chip technology to investigate antibiotic resistance and urinary tract infection (UTI) recurrence.

Two new tissue models have been developed to help researchers investigate UTIs in the bladder. The models provide a new insight into the behaviors of the causative bacteria, Escherichia coli (E. coli), that help infer them with antibiotic resistance, leading to UTI recurrence. The models were developed by researchers at the École Polytechnique Fédérale de Lausanne (EFPL; Switzerland) as part of the AntiResist consortium, which aims to deliver more representative in vitro disease models for the development of optimal treatment strategies.

What causes a UTI?

UTIs are typically caused by a subspecies of bacteria known as Uropathogenic E. coli (UPEC). These bacteria infect and multiply in the outer cells of the bladder epithelium known as umbrella cells. Once in place, these bacteria can form intracellular bacterial communities, which repeatedly rupture and reinfect bordering cells, leading to a reduction in the umbrella cells present and allowing further penetration into the lining of the bladder.

Why do UTIs recur?

Once buried deeper into the bladder epithelium, UPEC can establish quiescent intracellular reservoirs of bacteria. The bacteria in these reservoirs cease to proliferate and are protected from antibiotic attack, seemingly due to their location deep in the epithelial wall and quiescent cellular state.

These reservoirs can lay dormant during a course of antibiotics. When the immature bladder cells that the reservoirs preside in mature, rising to the surface of the epithelium, UPEC can then reinitiate growth, rupturing into the bladder lumen and leading to a second active infection [1].

Investigating UTIs

While there are indications for how these patterns occur, detailed examination of these mechanisms is challenging. Lead author of both studies, Kunal Sharma (EFPL), explained that “infection dynamics are difficult to capture from static imaging of tissue explants at serial time points. Thus far, in vitro models have not recapitulated bladder architecture with sufficient fidelity to study the time course of these events.”

To improve the models available to researchers and to answer their own questions about UTI infections, the team developed two in vitro models: a bladder organoid to accurately represent the tissue structure [2], and a bladder-on-a-chip to replicate the functional, physiological conditions of a bladder [3].

Applying the organoids

The organoid models were developed with fluorescence-labeled cell membranes, allowing the researchers to analyze them using live-cell confocal imaging. Using this method, the research team identified specific bacterial niches that developed within the models. Observations of multiple organoids revealed various patterns in host pathogen interactions that led to disparate outcomes for the infection.

Investigating how UTIs recur with organoid and organ-on-a-chip technology

Further analysis using volumetric electron microscopy, revealed that individual UPEC are able to rapidly infiltrate deep into the layers of the bladder epithelium, bypassing the need to form intracellular bacterial communities. These lone bacteria are, again, protected from antibiotics and immune cells due to their isolated position.

“This proof-of-concept system has shown promising potential for follow up studies on bacterial persistence to antibiotics and the dynamics of immune cell responses to infection,” remarked Sharma.

Bladder-on-a-chip insights

The bladder-on-a-chip models constituted human umbrella and bladder epithelial cells cultured together with a representative urine-flow system. Mechanical stresses akin to those experienced when a bladder fills and is emptied were applied to the model as the researchers examined the growth dynamics of UPEC over time.

This study revealed that neutrophils, recruited to the site of an infection, are incapable of eradicating intracellular bacterial communities. These same communities were found to be highly resistant to successive cycles of antibiotic treatment administered within the model.

Highlighting the utility of these models for investigating UTI recurrence and developing treatment strategies, senior author of both studies Vivek Thacker (EFPL) stated that “the two models complement each other well and are tailored to study specific aspects of the disease. We hope they will serve as a resource for the wider microbiology community and advance the synergies between the tissue engineering and infectious diseases communities.”

Taking synthetic organs from the lab to a chip: Anthony Atala and the advance of 3D cell biology

Anthony Atala, Director of the Wake Forest Institute for Regenerative Medicine (NC, USA), has had a long and illustrious career working in the development of lab-grown organoids to resolve the issue of long waiting lists for organ transplants. But Atala's work spreads far beyond this one field.

Here, Atala describes the changes he has observed in the field of regenerative medicine since his successful transplantation of a lab-grown bladder in 1999 – the achievement for which he is perhaps best known – and explores the challenges of developing these synthetic organoids. Having presided over the advance of several technologies to improve the production of organs, Atala is also well positioned to provide insights into the rapidly advancing field of 3D cell models. Discover his work in the development of new hydrogels and in the application of organ-on-a-chip models in this exclusive interview with BioTechniques.

Anthony Atala

Q) What are three key ways in which the field of lab-grown organs has changed over the last 22 years?

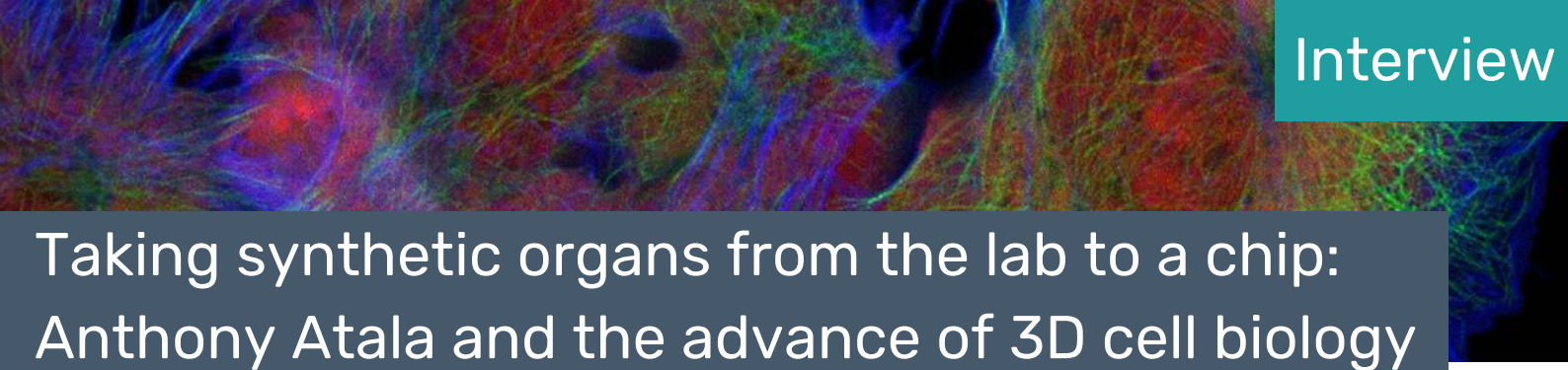
There are several things that have changed. First of all, back when we started, just growing cells outside the body was a major challenge — most human cells could not be grown or expanded ex vivo. The fact that we can now grow and expand human cells outside the body is a major advancement.

The second is raw materials. When this field was in its infancy, there were very few materials that had been developed to go inside the body and allow cells to grow and survive long term. This is another area that has changed tremendously; there are many different materials available now that enable us to do what is required.

Thirdly, our ability to three-dimensionally create these tissues with more advanced technologies, such as 3D printing, allows us to have greater precision. There are now over 40 different types of tissues and organs that are being developed in the laboratory. In the field, there is probably not a single organ in the body that someone has not tried to grow in the laboratory. The field has really changed, from its infancy to now, where there are attempts to create pretty much every tissue and organ in the body.

Q) Of those 40 plus tissues and organs that are in development, are there any that stand out as a significant achievement in the field?

One has to look at the complexity of the organs for that. When we look at human organs, we can determine that flat structures, such as skin, are the least complex. Tubular structures like blood vessels are the second level of complexity because the structures need to remain patent to allow air and fluid to flow through. The third level of complexity consists of tubular organs, such as the bladder or the stomach, mainly because you have more interaction with other organs and cells. And finally, the most complex are the solid organs, like the heart, liver and



Taking synthetic organs from the lab to a chip: Anthony Atala and the advance of 3D cell biology

lungs, mainly because there are so many cells per centimeter, that their vascular and nutritional requirements are very high.

Q) What are some of the techniques that are typically being used to develop lab-grown organs?

Much of what we're seeing right now in terms of organ development is focused on strategies that enable greater precision and scaling-up, and 3D printing is a technique that can be used to add those features to the field.

At our institute, we're using 3D printing to tackle the development of a number of organs, such as skin, tubular structures like blood vessels, urethras, hollow non-tubular organs and miniature solid organs.

With 3D printing, we can print both the cells and the scaffolds together. And while we print the cells and the scaffolds, we're also printing the channels to bring the vascularity or the blood flow into the construct.

Q) What kind of challenges does vascularization present?

That is still the major challenge. We need to make sure that the materials we print are able to maintain their structural integrity, whilst at the same time allowing for the vascularity for these channels to be formed and to remain open over time. Progress has certainly been made, but there is still a lot that remains to be done.

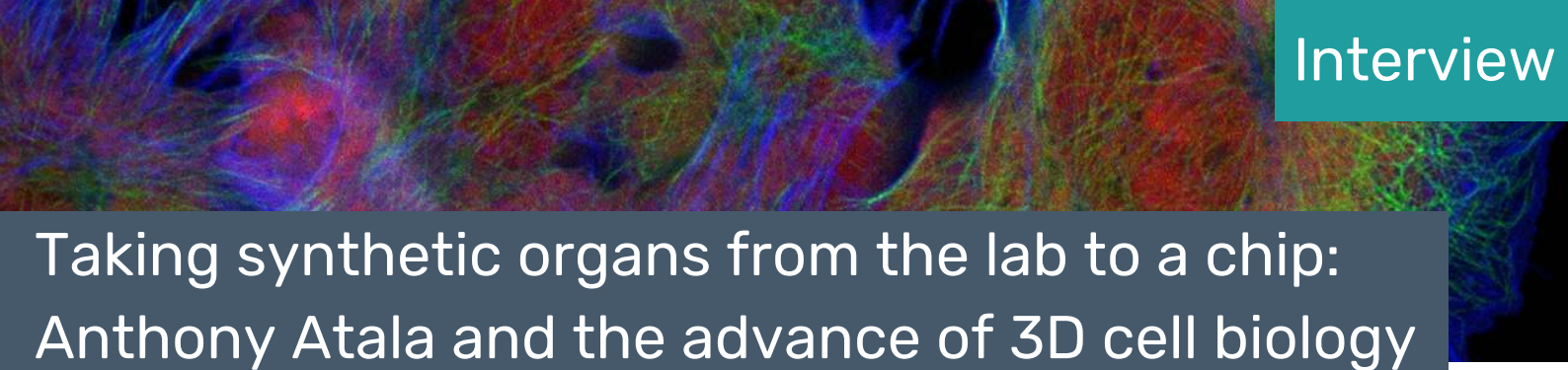
Q) Can you tell us about the achievement for which you were awarded the NASA vascular tissue challenge prize?

The NASA vascular challenge was created to see if it would be possible to create structures outside the body that were well-vascularized and had a critical dimension where you could keep the structure alive over a 30-day period. We entered the challenge with two different approaches.

One of the approaches was to create a vessel structure like the body creates, which is a tubular structure that feeds cells around it. But there's another way that the body gets vascularization: through spongy tissue, like the liver, for example. We entered the challenge with those two different approaches, trying to imitate what the body does. We were able to achieve the goals with both projects.

Q) How are you attempting to overcome difficulties associated with cell density and printing different cell types?

One of the major limitations is with solid organs. It's due to the fact that there are so many cells per centimeter that the nutritional requirements are very high and therefore it is very challenging to create solid structures that are functional and survive long-term. 3D printing allows nutrition to be brought to the central part of the construct being created, and this will hopefully lead to further advances in the development of solid organs.



Taking synthetic organs from the lab to a chip: Anthony Atala and the advance of 3D cell biology

Q) In a recent study a new synthetic hydrogel that can be successfully vascularized was created, will this development impact your work?

That's currently a major area of inquiry, to create better bio-inks and to create bio-inks that are tunable. One of the projects that we are working on is creating a universal bio-ink that you can tune up and down, depending on what tissue you are trying to print. We're working extensively to characterize bio-inks in a way that allows us to modulate these hydrogel scaffolds so we can use them for a wide variety of tissues.

Q) Is the primary focus of your organ generation to resolve the transplant issue, or can these organs also be used in drug discovery and development?

Yes, our primary focus has been to generate tissues and organs for human use. We are using the same 3D printing strategies that we use to create organs for patients to create what is called a body-on-a-chip, which are essentially miniature human organs. By creating these organs in a miniature manner, we can assemble multiple organs together on a chip device and then look at their interactions.

These systems can be used for drug screening and toxicity testing, to create disease models to test the effectiveness of treatments and for personalized medicine. For example, we can create tumor models, treat with different therapies and see which one has the best outcome. Studying the interactions between the organs provides more accurate insights into what the toxicity or therapeutic effects of a drug on a patient would be.

An example of this is when we used our organ on a chip device to test the drug bleomycin, which is a very effective chemotherapy agent but one that is toxic to the lungs. We found that, as expected, the drug was toxic to the lungs, but we also found that the miniature hearts that we had created stopped beating. We couldn't figure out why the heart stopped beating. We tried a new batch of hearts but they, too, stopped beating. We removed the livers as we thought it might've been due to a breakdown product of the liver, but the hearts still stopped beating.

We discovered that when bleomycin is toxic to the lung, the lung starts to secrete a protein that is damaging to the heart. We don't see this clinically because treatment is stopped before lung toxicity occurs. So potentially, by the time treatment is stopped, there may already be a degree of subclinical heart toxicity that we are unable to detect. We can use these models to determine the potential safety and efficacy of drugs before testing them in patients.

BioTechniques Spotlight

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Advanced Cell Models: Shifting Trends & Analysis

Last year we conducted a survey of our audience regarding 3D cultures. This year, we followed up on that survey and expanded it to include organ-on-a-chip technologies. This infographic reveals some of the shifting trends in the field of advanced cell models and their analysis.

Do you use advanced cell models in your work?

Yes	78.57%
No	21.43%

79% of respondents worked with Advanced cell models: an increase of 19% on last year's survey!

"And only 7% thought they would never use these models in their work!"

Do you think you will use advanced cell models...

In the next 3 months	57.14%
In the next 6 months	14.29%
In 6 months +	31.43%
Never	7.14%

Do you use one type of advanced cell model or a combination?

One type (organoid, spheroid, organ-on-a-chip)	42.31%
A combination	57.69%

Which kind of advanced cell model do you use predominantly?

Assembloids	17.86%
Organoids	42.86%
Spheroids	32.14%
Organ-on-a-chip	32.14%
Other	25.00%

Organoids had replaced spheroids as the most used models in this year's survey

Which disease areas do you use advanced cell models to study?

Cancer	31.82%
Neurodegeneration	18.18%
Developmental disorders	4.55%
Cardiovascular diseases	18.18%
Genetic diseases	13.64%
Infectious diseases	27.27%
Does not apply	18.18%
Other	9.09%

Unsurprisingly, the use of these models in infectious disease research had increased twofold on last year.

Which fields do you use advanced cell models to study?

Developmental biology	40.91%
Basic disease research	68.18%
Drug discovery and development	22.73%
Regenerative medicine	18.18%
Neuroscience	22.73%
Diagnostics	13.64%
Other	4.55%

Which organs/tissues do you use advanced cell models to study?

Womb	9.09%
Kidney	4.55%
Pancreas	4.55%
Does not apply	4.55%
Stomach	9.09%
Liver	9.09%
Lung	9.09%
Brain	31.82%
Cancer models	27.27%
Skin	13.64%
Intestine	13.64%
Heart	13.64%
Other	22.73%

What do your advanced cell models allow you to explore that you wouldn't be able to without them?

Areas of exploration

- Accurate study of the ECM
- Examine early development of tissues and diseases
- More ethical research
- Protein synthesis
- Multi-cellular responses to injury and infection
- To establish true cell structure and behavior in a tissue
- To test multicellular and multi-organ responses to novel therapeutics
- Reduced reliance on animal models
- Improved identification of therapeutic targets
- Spatiotemporal interactions
- Structure and flow. Endothelial and co-cultured networks in vitro.

Analyzing 3D cultures

Which of the below approaches do you use to analyze your 3D cell cultures?

Traditional microscopy	65.00%
Live-cell imaging	60.00%
Single-cell profiling/sequencing	25.00%
Spatial analysis	15.00%
CRISPR gRNA library screening	5.00%
Electrophysiological technologies	15.00%
Microelectrode arrays	15.00%
Microplate readers	25.00%
Does not apply	5.00%
Other	15.00%

Of the below, which do you see as the biggest advantage of 3D cell cultures?

Closer replication of in vivo conditions	70.00%
Speed of system establishment	0.00%
Ease of system maintenance	10.00%
Ease of system scalability	0.00%
Ease of genetic manipulation	0.00%
Personalization	15.00%
Does not apply	5.00%

Unsurprisingly, recapitulation of ***in vivo*** environments were seen as the biggest advantage of advanced cell models.

Which of the below do you see as the greatest challenge of working with 3D cell models?

Standardization	10.00%
Reproducibility	35.00%
Expense	25.00%
Establishing an accurate microenvironment	20.00%
Establishing large scale models	10.00%

Of the below, what is the biggest challenge in the analysis of 3D cell cultures?

Culture size	5.56%
Examining the interior of the structure	27.78%
Passaging cultures	5.56%
Combining cultures of different tissue/cell types	55.56%
Other	5.56%

What technologies or platforms do you use for high-throughput and easy data analysis of 3D cell cultures?

Light microscopy techniques
Fluorescent imaging and bioluminescent cell health assays
Incucyte Live Cell Analysis System
Multi-mode plate reader
High content image analysis and Matlab script analysis of the data
Epifluorescent & immuno-histology
Classical histology & classical biochemistry
PCR

What do you see as the biggest advantage of organ-on-a-chip models?

Mimicking the micro-environment of tissues and organs	52.63%
Increased kinetic control of experimental conditions	21.05%
Cost	10.53%
Ease of use	0.00%
Does not apply	10.53%
Other	5.26%

What do you consider the greatest challenges of working with organ-on-a-chip models

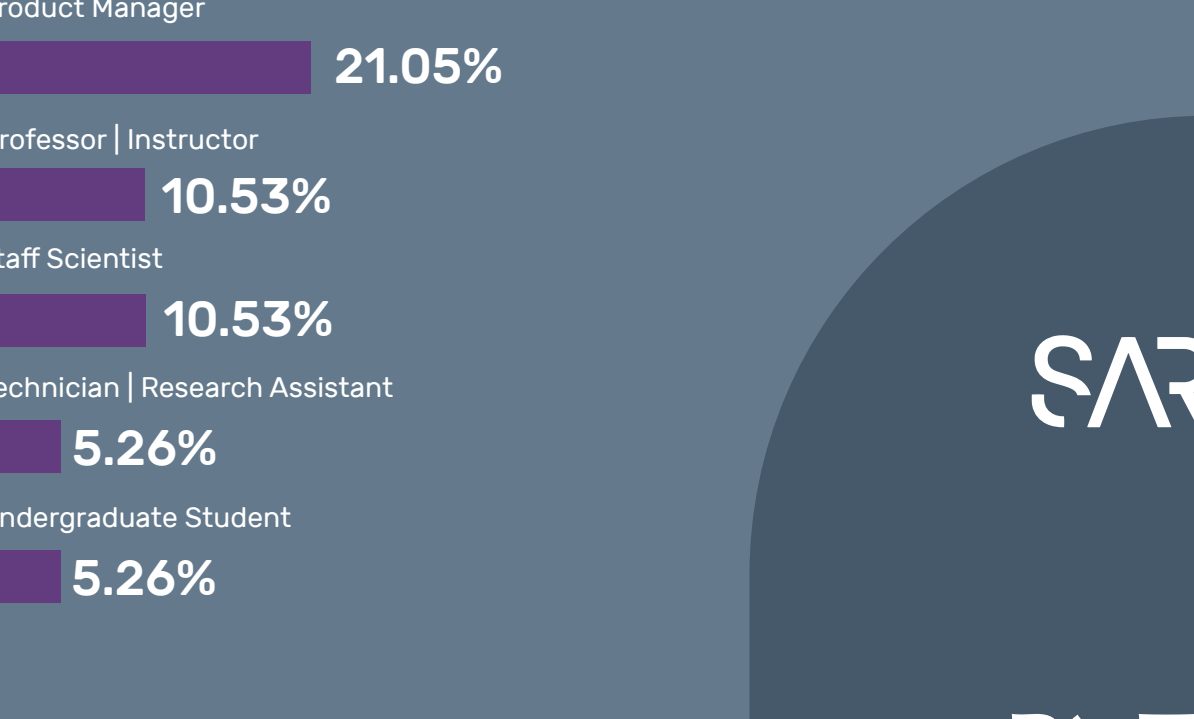
Surface effects of microfluidics	10.53%
Minimal mixing of study reagents and liquids	0.00%
Achieving high-throughput screening	15.79%
Scaling up	21.05%
Setting the correct arrangement to replicate tissue/organ interactions	36.84%
Does not apply	10.53%
Other	5.26%

What type of advanced cell model most excites you?

Assembloids	11.76%
Organoids	35.29%
Spheroids	5.88%
Organ-on-a-chip	29.41%
Other	17.65%

About the respondents

Location



- 13.33% Canada
- 13.33% Swizerland
- 33.33% United States
- 6.67% Mexico
- 6.67% Luxembourg
- 6.67% Spain
- 6.67% Philippines
- 13.33% Australia

Which of the following best describes your job title?

Department Head	6.67%
Graduate Student	6.67%
Lab Director | Chief Scientist	6.67%
Medical Profession | Physician	13.33%
President | CEO | VP | Owner	10.53%
Principal Investigator	5.26%
Process Engineer	52.63%
Product Manager	21.05%
Professor | Instructor	10.53%
Staff Scientist	10.53%
Technician | Research Assistant	5.26%
Undergraduate Student	5.26%

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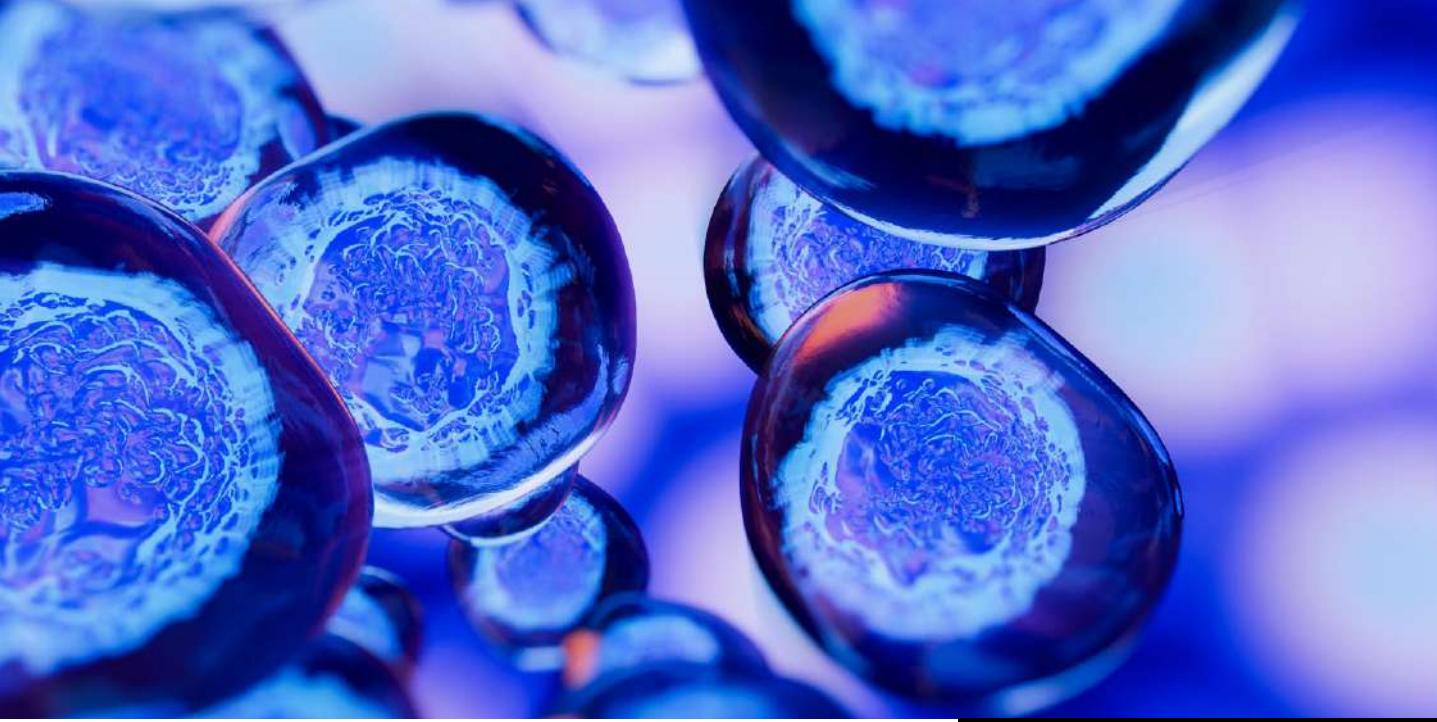


Organoid Analysis Guide

Simplifying Progress

SARTORIUS

Introduction

Organoids are rapidly becoming a powerful tool for both basic research and drug discovery, spanning a wide range of biomedical applications including oncology, regenerative medicine, disease modeling, and drug screening. These three-dimensional (3D) organotypical structures can be grown *in vitro* to produce miniaturized versions of the organs from which they were derived. The starting point for development of organoid cultures can be tissue-specific, resident adult stem cells, cancer stem cells derived from patient biopsies, or pluripotent stem cells, either embryonic or induced. When established with 3D extracellular matrices, the cultures can recapitulate the *in vivo* architecture, spatial organization, and genetic diversity of the cell populations found in the original organ with remarkable fidelity.[1] As self-organizing and self-renewing 3D structures, organoids offer a distinct advantage over traditional monolayer culture techniques and offer a more physiologically relevant milieu in which to understand complex biology with greater clarity.

In this eBook, we explore some of the translational applications of organoids, highlight key considerations for successful 3D culture and expansion *in vitro* and describe leading-edge technology for the imaging and objective analysis of these complex, self-organizing tissues.

Translational Applications

The versatility of organoids, including their ability to be cultured from both healthy and diseased cells and tissues has unlocked a remarkable breadth of research and medical applications. While many of these applications are still in the early stages of development and use, the potential to reveal mechanisms of disease with greater precision and detail and enable more personalized approaches to disease treatment continues to accelerate progress in the field. Undoubtedly, human organoids will provide new opportunities for the study of disease and offer an important complement to existing cell line and animal models. Several recent review articles provide an in-depth look at the development and use of organoid-based disease model systems.[1, 3, 4, 5, 6]

Hereditary Disease Modeling

Because organoids retain the genetic signatures of the tissue from which they are derived, they are well-suited for modeling genetic diseases such as cystic fibrosis (CF), the first human disease to be modeled using organoids.[7] CF is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTCR) gene which encodes a chloride channel protein involved in the regulation of adsorption and secretion of salt and water. The mutations affect epithelial cells in a variety of organs including the lungs and intestines, leading to a build-up of viscous, sticky mucous and infections in the lungs and chronic digestive system problems. Organoids derived from CF patients have continued to evolve and are now used to predict which drugs are most effective against different mutations[8] and to monitor drug levels in the blood of patients as an alternative to conventional pharmacokinetic testing.[9]

What's in a Name?

An organoid should satisfy several criteria[2]

- Possess a 3D structure containing cells that establish or retain the identity of the organ from which they were derived
- Include the presence of multiple cell types, as in the organ itself
- Exhibit some aspect(s) of the specialized function of the organ
- Display self-organization according to the same intrinsic organizing principles as in the organ itself

Organoids have also been developed as models for other genetic diseases including alpha 1-antitrypsin (A1AT) deficiency[10], primary microcephaly[11] and Leber congenital amaurosis[12] which affects the retina and leads to blindness. These examples demonstrate the valuable contribution that organoids offer to the discovery and development of new therapeutics by enabling creation of patient-derived, physiologically relevant models of disease.

Host-Pathogen Interactions

Organoids are also being used to model infectious diseases affecting humans. A major advantage offered by organoids for the exploration of host-pathogen interactions is they can contain virtually all of the cell types present in the organ from which they were derived. As a result, they can closely recapitulate the *in vivo* environment with which a pathogen interacts and causes damage. Organoids have been used to model Salmonella infections in gallbladder[13], to study cholera toxin inhibitors in the intestine[14] and Helicobacter pylori infection in the stomach.[15]

Forebrain-specific organoids derived from human iPSCs have been used to model the microencephaly associated with ZIKA virus infection during pregnancy. The organoids recapitulate key features of cortical development and ZIKA infection of the organoids leads to growth inhibition and increased apoptosis, implying that the virus alters neurogenesis during brain development which is causative to microencephaly.[16]

Oncology

Cancer-derived organoids are providing researchers and drug developers with a powerful new tool to both better understand the complexities of this disease and develop more effective treatments. Organoids can be efficiently generated from tumor tissue, are relatively easy to propagate and represent the transcriptional and mutational profile of the original tumor.[17] They also overcome the challenges presented by cancer cell lines which accumulate additional mutations over time and animal models which do not fully reflect the genetic characteristics of human cancers.[1] Tumor organoids also address key shortcomings of patient-derived tumor xenografting (PDTX). While PDTX more faithfully recapitulates the original tumor, the process is labor-intensive, time-consuming, expensive and not amenable to high-throughput screening.

The potential of patient-derived tumor organoids spans several applications.[18] Organoids from a diverse set of cancer types can be used to create a biobank and then combined with extensive genome sequencing, expression profiling, drug sensitivity and patient clinical data. Access to such an unprecedented repository of information will certainly aid and accelerate research and the search for new therapeutic candidates by facilitating large scale screening efforts. These models can also be used to compare *in vitro* and *in vivo* treatment response; because organoids can be cultured indefinitely, *in vitro* dosing regimens can be varied so that the outcome aligns with that observed in the clinic. Ultimately,

this knowledge can help determine whether standard of care treatment responses of organoids are able to predict the response of patients to treatment. Finally, patient-derived tumor organoids can be used to select specific treatment; this ability will be critical as a growing array of molecular targeted agents are becoming available.

Regenerative Medicine

Given the ability of organoids to include the cellular lineages present in the organ from which they are derived, they have the potential to serve as an unlimited source for replacing damaged tissues. This approach has been demonstrated using mouse colon organoids expanded ***in vitro*** from a single stem cell. When the organoids are transplanted into mice with damaged colons, functional crypt units were restored, and epithelial barrier function was fully recovered.[19]

Restoration of function may also be possible by combining organoid technology with gene therapy. Transplantation of organoids in which genetic mutations have been repaired using gene editing technologies could potentially be exploited to treat patients with the disease. As a proof of concept for gene correction in patients with a single-gene hereditary defect, CRISPR/Cas9 gene editing was used to correct a CFTR mutation in small intestinal and rectal organoids from two CF patients; the corrected allele was shown to be fully functional.[20]

Organoid Culture and Analysis

As self-organizing and self-renewing structures, organoids have a distinct advantage over traditional monolayer culture techniques and hold unprecedented potential for many applications as described above. Adding to their value as a basic and translational research tool is the fact that traditional cell culture techniques and technologies can be used to initiate and expand *in vitro* cultures and maintain *in vivo*-like characteristics, tissue-specific functions and disease-state phenotypes. An informational guide to general organoid culture conditions is available from the American Type Culture Collection (ATCC) and provides guidance on initiation, expansion and cryopreservation.[21] An extensive set of resources and methods is also available on the StemCell Technologies website.[22] In addition, several review articles have compiled comprehensive lists of protocols developed for tissue-specific stem cell- and patient tumor-derived organoids.[1, 5, 23]

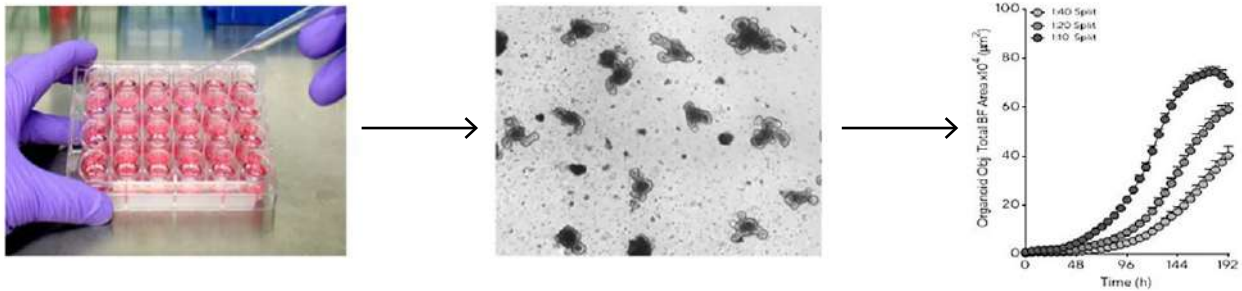
While protocols that define culture conditions, seeding densities, matrix recommendations and other critical parameters have been defined for organoids from many types of normal and diseased tissue, quantitative analysis of these differentiated micro-tissues has been challenging. Currently, characterization and optimization of organoid cultures are limited in their ability to objectively monitor these 3D structures as they form and grow over time. Specific shortcomings include:

- A lack of validated protocols for reproducible well-to-well organoid formation
- Low data throughput
- Time-consuming and manual processes for acquisition of organoid images
- The need for third party analysis software, with limited quantitative information
- Loss of environmental control during image acquisition which may introduce variability in assay results

While organoids may be established using familiar cell culture tools and technologies, their complexity has driven the need for sophisticated, quantitative analytical methods. The need for an unbiased, quantitative understanding of the dynamic intercellular relationships within organoids has been described as ***"essential to fully comprehend the complexity that can be modeled with these systems."***[24]

Real-Time Live-Cell Analysis of Organoid Cultures

Quantitative, label-free *in vitro* approach enabled by the Incucyte® Live-Cell Analysis System and organoid analysis software are becoming a requisite for the study of organoids in foundational research, disease modeling and drug screening. This powerful end-to-end platform offers the ability to kinetically visualize and quantify the formation and growth of organoids cultured in Matrigel® domes (Figure 1).



1. Prepare assay plate

Pipette Matrigel® containing organoid cells or fragments into the center of the well of a 24-well or 48-well plate to form a dome.

2. Acquire images

Capture images over time in a physiologically relevant environment.

3. Analyze organoid

Organoid size, count, and morphology can be assessed using brightfield imaging and segmentation enabling label-free analysis.

Figure 1: Established protocols and Incucyte® analysis tools enable unbiased quantitative characterization of organoids and optimization of culture conditions.

Proprietary brightfield (BF) image acquisition enables real-time kinetic imaging of 3D organoids with size, count and morphology measurements automatically plotted over time, providing quantitative data on differentiation and maturation characteristics.

Figure 2 summarizes the organoid culture quality control workflow. Detailed protocols for the following experiments are available in the Sartorius application note entitled Real-Time Live-Cell Analysis of 3D Organoid Growth in Matrigel® Domes.[25]

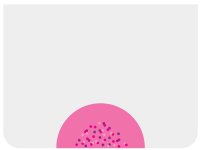
1. Harvest and resuspend organoids in Matrigel®

Harvest and resuspend organoid cells or fragments in Matrigel® (50–100%).

2. Pipette Matrigel® in plate

Pipette Matrigel® containing organoid fragments in the center of a 24-or 48-well plate (30–50 µL or 10 µL, respectively).

3. Polymerize Matrigel®



Incubate plate at 37° C for 10–15 mins to polymerize Matrigel®, forming a dome.

4. Add media

Overlay polymerized dome with recom-mended culture media.

5. Monitor organoid formation and growth

Place plate in Incucyte® to monitor organoid formation and growth.

Figure 2: Incucyte® lab tested protocol for culturing organoids in Matrigel® domes. Protocol allows easy culture, expansion and maintenance of organoid cultures for representation of organ-specific physiology.[26]

Monitoring and Quantifying Organoid Growth

Figure 3 demonstrates the ability of the Incucyte® Organoid Software Analysis Module to visualize individual organoids embedded throughout the Matrigel® dome. The top row shows a single, in-focus BF 2D image for the three different types of organoids, six days post seeding; the middle row shows enlargements of the boxes in the top row, revealing cell type-specific morphological features. Time-course plots (bottom row) showing individual well total BF area (µm²) over time, demonstrating cell-type specific organoid growth. Mature hepatic and pancreatic organoids showed similar size and rapid growth while the intestinal organoids appeared smaller and exhibited a distinct budding phenotype as they mature.

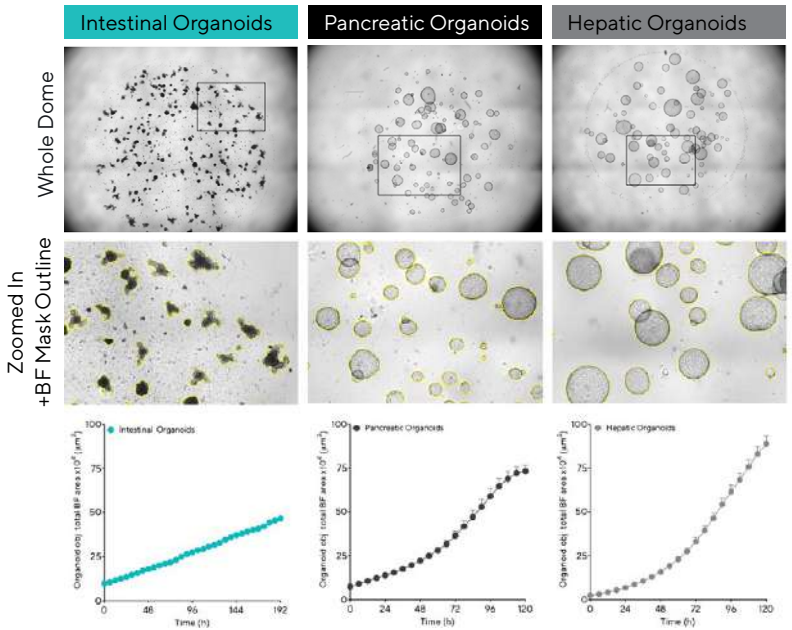


Figure 3: Acquisition and quantification of organoid growth in Matrigel® domes. Mouse intestinal (1:3 split, 50% Matrigel®), pancreatic (1:5 split, 100% Matrigel®) and hepatic organoids (1:40 split, 100% Matrigel®) were embedded in Matrigel® domes in 24-well plates and imaged every 6 h in an Incucyte® system. All images captured at 4X magnification. Each data point represents mean ± SEM, n = 4 wells.

Optimizing Organoid Culture Conditions

Defining optimal culture conditions is essential for establishing healthy organoids for use in downstream studies. Characterization of organoid formation using real-time kinetic measurements on the Incucyte® platform provides the ability to objectively assess the impact of growth conditions and seeding densities and define the conditions necessary to maximize expansion.

Figure 4 summarizes a study used to determine optimal conditions for maximal expansion of mouse hepatic organoids seeded at multiple densities in Matrigel® domes. Organoid size and count were automatically analyzed by the Incucyte® software. BF images taken five days post seeding and time-course plots demonstrated that organoid size and growth rate was directly proportional to cell number. Organoids seeded at the highest density appeared larger (> 500 µm diameter) and exhibited rapid growth reaching maximal size within 120 hours (BF images and total area time-course, respectively). At lower densities, the organoid maturation phase was extended, and the greatest growth potential (size) was observed (average area time-course).

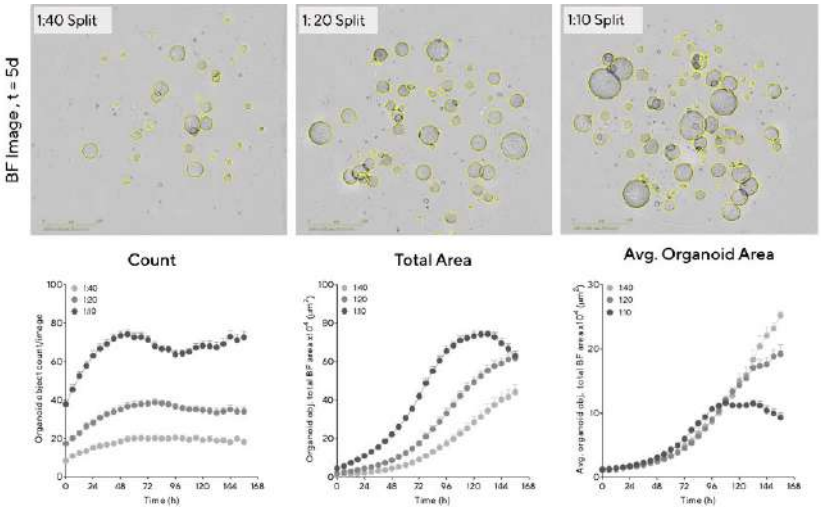


Figure 4: Determine optimal conditions for maximal organoid expansion. Mouse hepatic organoids were embedded in Matrigel® domes (100%) in 48-well plates at multiple seeding densities. BF images were taken five days post seeding. Data were collected over 168 h at 6 h intervals. All images captured at 4X magnification. Each data point represents mean ± SEM, n = 14 wells.

Defining the Optimal Organoid Maturation Phase

Morphology metrics are used to determine the appropriate passaging frequency for organoid cultures. These metrics are cell-type specific and include indicators of maturation such as budding and accumulation of debris within the organoid lumen. Real-time tracking of changes in organoid eccentricity (object roundness) and darkness (object brightness) with the Incucyte® platform enables rapid, unbiased assessment of optimal culture passage periods.

To define the optimal passaging frequency for mouse hepatic organoids embedded in Matrigel® domes, cultures were imaged using the Incucyte® platform for eight days (Figure 5). Hepatic organoids are typically ready for passaging when the majority of organoids have reached their maximum growth and have not collapsed.[27] Representative BF images show that two days post seeding, cultures were not ready for passaging as the majority of organoids were less than 100 µm in diameter and had clear lumens. A decline in eccentricity was observed within 48 hours as organoids formed and became more rounded (time-course data; far right panel). The optimal period for passaging this culture occurred between days four and five, when most organoids within the dome had reached maximal size, exhibited a rounded morphology and had not collapsed. The image and time-course data from day six indicated that the organoids which had reached maximal size had collapsed and darkened.

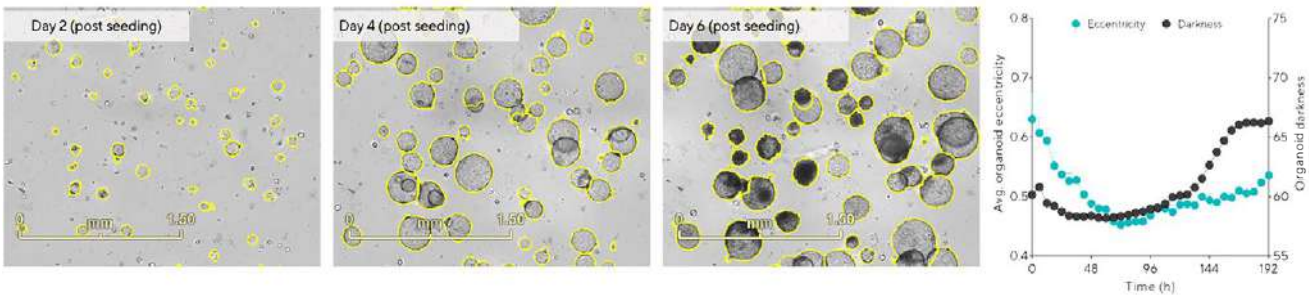


Figure 5: Define cell-type specific passage frequency using integrated morphology metrics. Hepatic organoids were embedded in 100% Matrigel® domes (1:10 split) in 24-well plates. Data were collected over 192 h at 6 h intervals. All images captured at 4X magnification. Each data point represents mean ± SEM, n = 6 wells.

Tracking Organoid Differentiation and Growth Efficiency

Under routine culture conditions, organoid morphology and growth capabilities are expected to remain consistent across multiple passages. Monitoring of key parameters using qualitative approaches to confirm this, however, can be time- and labor-intensive and introduce bias.

In contrast, the Incucyte® platform can be used to kinetically and objectively quantify multiple parameters to assess organoid expansion and growth efficiency during extended passaging (Figure 6). When maintained at a consistent density, intestinal organoids exhibited comparable count, area, eccentricity, and darkness measurements over time. In addition, representative BF images at Day 7 post seeding confirmed the maintenance of a distinct budding phenotype. These results demonstrate the ability of this imaging and analysis approach to support robust and reproducible assessment of long-term organoid expansion.

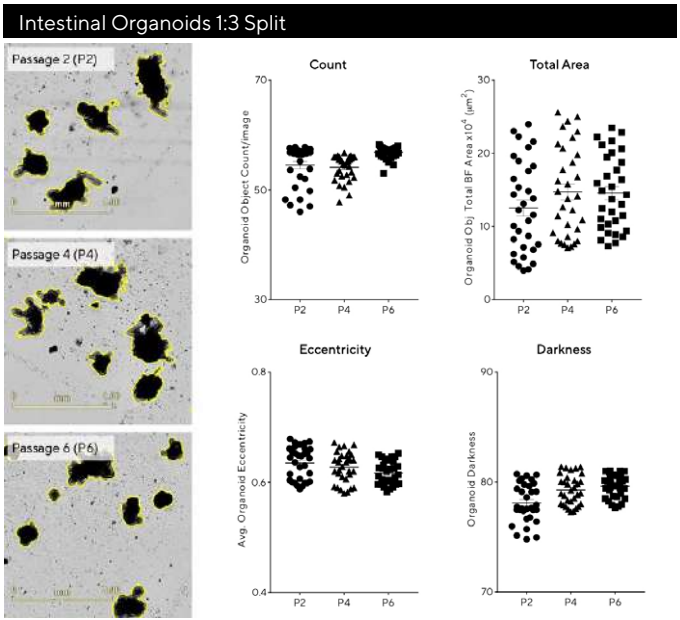


Figure 6: Assess growth and differentiation efficiency of organoids across multiple passages. Intestinal organoids were embedded in 50% Matrigel® domes (1:3 split, 24-well plate) over multiple passages and evaluated for growth and differentiation consistency over time. Data were collected over 192 h at 6 h intervals. All images captured at 4X magnification. Each data point represents mean ± SEM, n = 6 wells.

Unlocking the Potential of Organoids

The ability to establish organoids from human progenitor cells has ushered in a new era in both fundamental and translational research. As self-organizing tissues, organoids can recapitulate organ architecture, offering a more physiologically relevant *in vitro* model of normal and disease processes. While detailed protocols exist for establishing organoid cultures for dozens of tissues and cancers, technological advancements are essential to enable rapid, accurate and unbiased imaging and analyses. Neither qualitative assessments nor techniques used with 2D monolayer cell cultures offer the ability to confidently determine the health and quality of these complex 3D tissue structures—which can impact the accuracy and reproducibility of downstream assays.

The Incucyte® Live-Cell Analysis system, in combination with the Incucyte® Organoid Analysis Software Module, facilitates kinetic, qualitative and unbiased evaluation of organoid formation and growth. This breakthrough technology for organoid imaging and objective analysis provides important advantages including the ability to:

- Automatically analyze 3D organoids embedded within Matrigel® domes
- Use integrated, real-time label-free metrics to optimize and define culture conditions and regimes
- Determine the optimal periods for passaging or extension of organoid cultures based on integrated morphological parameters
- Assess culture quality during extended passaging

Undoubtedly, human organoid systems will continue to improve and provide unprecedented opportunities to better understand complex disease mechanisms. The ability to objectively define parameters such as seeding density, passage frequency and morphology will be critical to establishing healthy cultures for use in downstream studies and unlocking the full potential of organoids.

The ultimate goal of *in vitro* 3D cell culture is to enable more physiologically relevant downstream analyses of cells. Through comprehensive characterization, scientists can develop a deeper understanding of biology, ensure more robust maintenance of stem cells during extended cultures, and monitor cells at every step of the 3D organoid workflow. A key element in controlling adverse variables is to standardize workflows (including organoid culture QC and assay steps) and metrics, thereby eliminating human subjectivity and interpretation.

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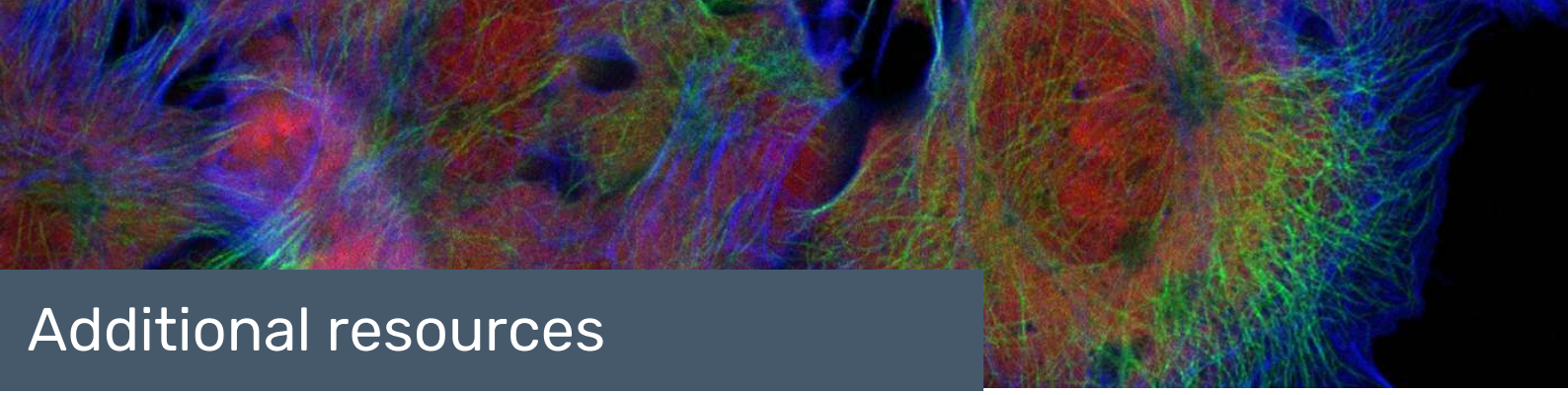

Additional resources

You can find more resources, including guides, Application Notes and further eBooks, in our Spotlight on advanced cell models, produced in association with Sartorius. Check out these key highlights below.

Panel Discussion: The evolution of advanced cell models and their analysis

This panel discussion evaluates the shifting trends in advanced cell models, examining their advancement and increasing prevalence in the lab. Panelists also discuss the challenges presented in the analysis of these complex technologies and reveal top tips for obtaining the best results from each type of model.

eBook: Organoids in drug discovery

Using 3D model systems requires advanced tools for monitoring cell growth and performing complex real-time assays. This eBook focuses on the utility of organoids in drug discovery, and how advanced live-cell analysis platforms allow for real-time monitoring of cell health, behavior and morphology of complex models.

Application Note: Real-time live-cell analysis of 3D organoid growth in matrigel domes

Recent advances in organoid technology have opened up new horizons for translational human disease research, disease modeling, regenerative medicine and predictive precision therapies. This Application Note presents a solution for specific and reliable in-vitro analysis, standardizing and automating organoid culture workflows and simplifying culture characterization and optimization.

BioTechniques Spotlight

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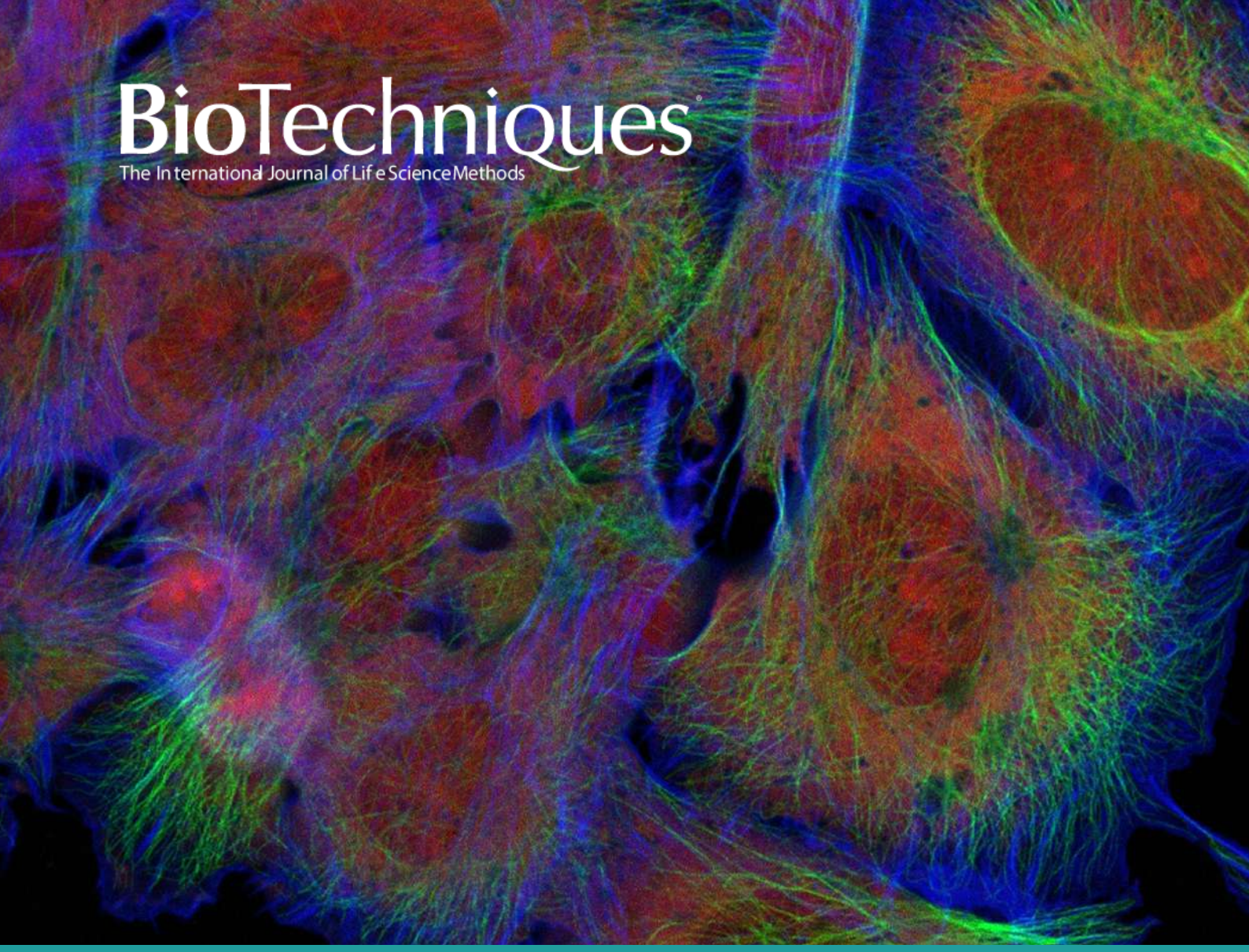

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