BEST PRACTICE EBOOK

Optimising Cell Culture

by Tackling Translational Barriers through Automation and 3D Models

Explore how industry experts and top academics are addressing shortcomings of traditional cell culture

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Introduction

Cell culture is a key area of biomedical research and therapeutic development that advances our understanding of biological processes, disease mechanisms, and drug discovery. It involves growing cells from animals or plants in an artificial environment, where maintaining high standards for quality reagents and equipment calibration is essential. Automation and specialised reagents play a critical role in ensuring the optimal conditions for cell growth.

Advanced techniques, such as 3D models and microphysiological systems (MPS), provide more accurate simulations of in vivo environments than traditional 2D cell culture. The physiological relevance of 3D models allows them to reliably reproduce tissues and tumours, this accuracy can facilitate and drive forward research in fields like cancer treatment and regenerative medicine.

Cell culture models have been used to study human health and disease for a long time, yet reproducibility, scalability and contamination remain challenging. In cell culture assays, a large proportion of reproducibility issues arise from the biological variation between generations of cells. The surface area of the vessels needed to grow large quantities of cells increases significantly as the scale of cell production rises. This expansion impacts the lab's capacity and resources, requiring more incubators to accommodate the larger surface area. Additionally, more operators are needed to manage the increased workload, which results in higher labour demands and increased operational costs. Any breach in sterility can lead to contamination which can lead to inaccurate results and misinterpretation.

This eBook offers detailed insights on best practices, focusing on reproducibility, reliability, and scalability. By adhering to these practices, scientists can drive innovation and continue pushing the boundaries of cell culture research and development.

Lucia Simmen
Digital Content Editor, Oxford Global

Contents

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- Kyoto University Hospital Explores the Potential of Treating Type 1 Diabetes Using Induced Pluripotent Stem Cells
- Historic Islet Cell Transplant Offers Hope for a Permanent Cure for Type 1 Diabetes
- Is Single-Use Bioprocessing the Way Forward?

Key Summaries

PEDRO PINTO
Lab Head,
University of Greifswald

Pedro Pinto of the **University of Griefswald** highlights how microphysiological systems (MPS) is an advanced method for modelling an in vivo environment. Due to the complexity of certain organs and tissues it can be challenging to recapitulate their structure and behaviour in vivo. However, Pinto's MPS study gives crucial insights into cancer progression and cellular responses.

SIVANE KOSKAS
Global Product Manager
Advanced Instruments

Sivane Koskas from A**dvanced Instruments** discusses how Advanced Instruments' Solentim Ecosytsem adopts best practices such as automation, specialised reagents and high-quality imaging programs to tackle several major challenges in cell line development (CLD). The principal challenges in CLD are maintaining pluripotency in stem cells, fostering clonal growth expansion and single-cell isolation.

PAULA MELEADY
Associate Professor,
Dublin City University

Paula Meleady of **Dublin City University** demonstrates that proteomics and ubiquitination are key to optimising cell lines like Chinese hamster ovary (CHO) cells. Biotherapeutics are expensive and not all patients have access to them. Dr Meleady shows how analysing a CHO cell line's proteome gives insight into factors affecting production efficiency. Understanding ubiquitination allows scientists to develop more stress resistant cell lines which could increase the efficiency of biopharmaceutical production, potentially reducing costs and speeding up the availability of new therapeutics.

JOSH BAGLEY
Chief Scientific Officer,
a:head Bio

Josh Bagley of **a:head Bio** explores how to overcome the translational gap (disconnect between clinical and preclinical research) in the field of central nervous system (CNS) therapeutics. Bagley proposes that developing brain organoids is key since they can recapitulate the complex neuronal networks present in the brain. Given that the human brain is highly complex and different to an animal one, the nature of brain organoids makes them more appropriate for studying human disease and CNS therapeutic intervention than animal models.

TAMARA ZIETEK
Chief Executive Officer,
Doctors Against Animal Testing

Tamara Zietek of **Doctors Against Animal Experiments** examines how human based models including organoids are much more efficient than animal models at mimicking human organs. Yet many companies and regulatory bodies still rely on data from animal models. Zietek suggests that high quality data and imaging systems will help build trust and reliability in human models and encourage wider uptake across the biopharma community.

Overcoming Limitations in Cancer Research: Advancing Kidney Cancer Models with Microphysiological Systems for Better Disease Insights

Pedro Pinto, Lab Head at the University of Greifswald explored the application of microphysiological systems (MPS) in cancer research, the focus was on creating complex in vitro kidney cancer models. The presentation highlighted the limitations of conventional in vitro models like monolayer cultures, spheroids, and organoids, and introduced MPS (or organ-on-a-chip) as an advanced alternative for simulating the in vivo environment more closely. These systems incorporate additional stimuli like shear stress, pressure, and flow to better mimic physiological conditions.

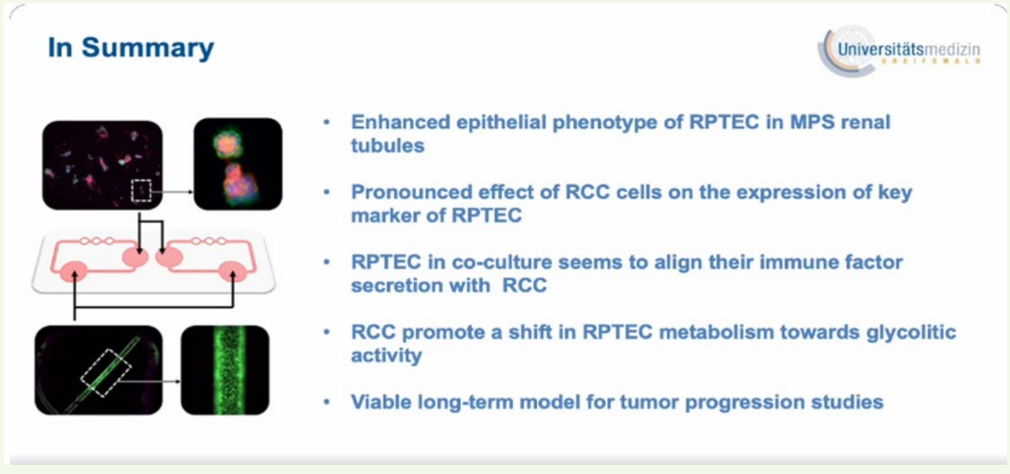
Pinto described using a specific MPS platform that contains microfluidic channels and culture chambers, which enables the study of interactions between healthy kidney cells and renal carcinoma cells. The developed model consists of a renal tubule and cancer spheroids in agarose gels. This setup allows for extended dynamic culture periods and prevents direct contact between the cells, while still allowing them to share media.

In the MPS, healthy renal tubules showed enhanced polarization and mitochondrial energy production, indicating a closer resemblance to actual kidney physiology compared to traditional 2D cultures. This demonstrates the MPS physiological relevance and value.

His findings also demonstrated that when healthy kidney cells were exposed to cancer cells, they altered their gene expression, secretion of immune-related factors, and metabolism. For example, the study found upregulation of markers like cytokines and changes in glucose metabolism in healthy cells due to the presence of cancer cells.

Furthermore, the system was used to study how the COVID-19 spike protein affects kidney cells with or without cancer. The results indicated an amplified immune response in the presence of both cancer and spike protein, suggesting that cancer may exacerbate immune reactions during viral infection. Additionally, by incorporating immune cells into the MPS, researchers observed immune cell recruitment by the cancer spheroids, though they found limited cancer cell death in their setup. The team also explored the transformation of healthy cells into a cancer-like phenotype, using microRNA profiling to identify potential signatures of this process.

In conclusion, the MPS model developed by Pinto and his team demonstrates enhanced epithelial behaviour in kidney cells under flow conditions and dynamic changes in cell activity when influenced by cancer cells. The platform also allows for long-term studies, providing valuable insights into cancer progression and cellular responses.



Automated Solutions for Overcoming Key Challenges in Cell Line Quality & Clonal Growth

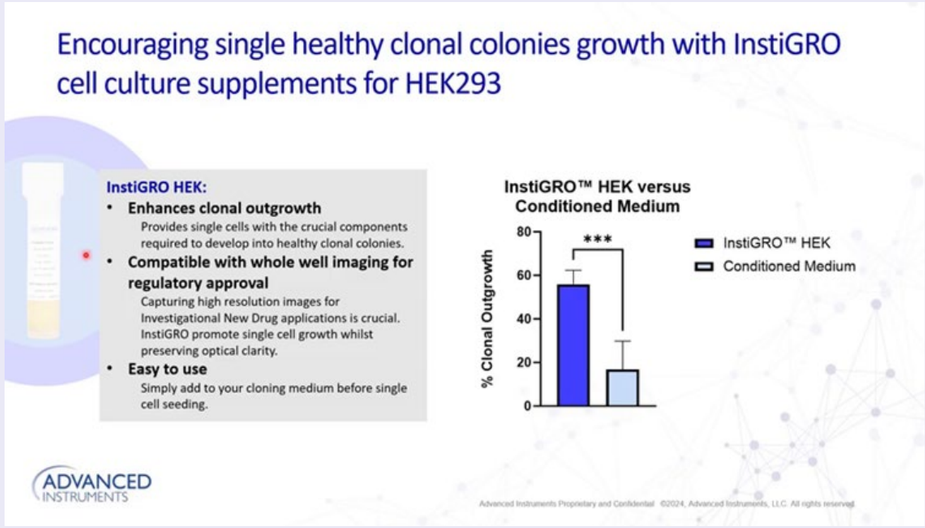
In this presentation, **Sivane Koskas, Global Product Manager at Advanced Instruments** outlined best practices in cell culture and quality control, particularly focusing on the challenges of developing cell and gene therapies. These best practices are highlighted through four key challenges in cell line development (CLD), each addressed with specific solutions offered by Advanced Instruments' solentim ecosystem. The presentation stressesd the following points:

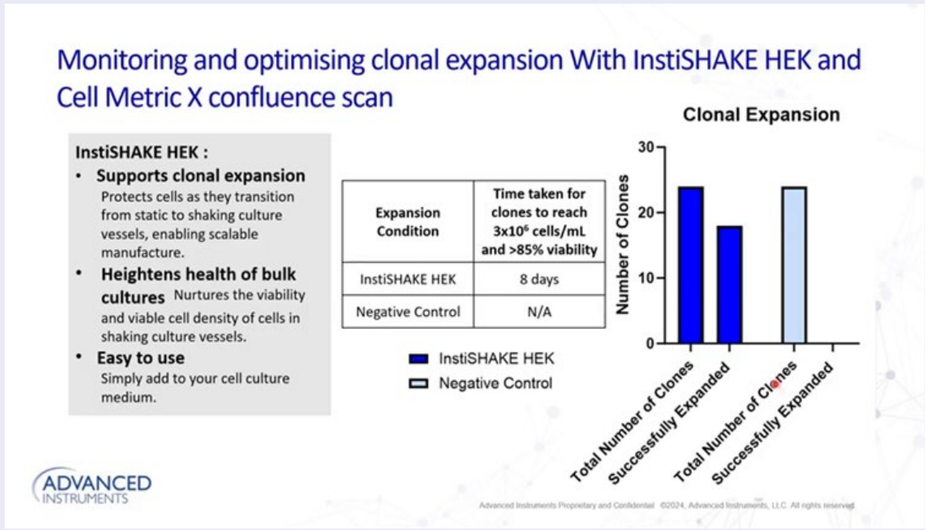
Single-cell isolation and clonality assurance: A critical challenge in CLD is ensuring that a single cell is isolated and confirmed as monoclonal, as required by regulatory bodies. Koskas introduces the Solentim VIPS Pro, which automates single-cell seeding and whole-well imaging to capture high-resolution images for clonality proof. This reduces manual errors and speeds up the isolation process, ensuring precision and compliance with regulatory standards.

Encouraging clonal growth: Another challenge in CLD is promoting the growth of isolated cells into healthy clonal colonies. Advanced Instruments' InstiGRO supplement provides essential nutrients in a chemically defined, optically suitable form that supports cell growth and reduces variability seen in conditioned media. This ensures higher efficiency in cloning, thereby optimising resource usage and minimising plate requirements.

Maintaining pluripotency in stem cells: Maintaining pluripotency in induced pluripotent stem cells (iPSCs) is taxing but Advanced Instruments offers solutions like Matrigel and laminin, which support iPSC growth while maintaining their stem cell characteristics. These matrices are imaging-compatible, making it easier to track cell behaviour and pluripotency during development.

Monitoring and optimising clonal expansion: Expanding isolated clones into large colonies while maintaining cell viability and productivity is another challenge. The Cell Metric uses AI-driven scanning technology to monitor colony expansion, providing highly precise measurements of confluence, which is essential for scaling up production. The solution reduces the time spent manually inspecting colonies, increasing efficiency.





Overall, Koskas presented an innovative approach to overcoming the challenges of cell culture. She emphasised that automation, precision, and the use of specialised reagents improve cell line development and quality control. These best practices help streamline biopharmaceutical production, improve scalability, and ensure compliance with regulatory standards.

Exploring the Potential of Protein Degradation Pathways to Optimise CHO Cell Lines & Enhance Biopharmaceutical Efficiency

Dr. Paula Meleady, Associate Professor at Dublin City University focused on optimising Chinese hamster ovary (CHO) cell lines for efficient biopharmaceutical production and understanding cellular stress mechanisms. The global biopharmaceutical market, which includes products like vaccines, monoclonal antibodies, and recombinant proteins, is projected to reach $800 billion by 2030. CHO cells are the primary workhorses for producing over 70% of these drugs.

The aim of her research is to optimise CHO cell productivity, thereby reducing the cost of biotherapeutics. This would ultimately make therapeutics more accessible to patients. The research involves a systems biology approach to understand and improve CHO cell processes at the genomic, transcriptomic, proteomic, and epigenomic levels.

Dr. Meleady's research has focused on analysing CHO cells' proteome to explore factors affecting production efficiency. Recently, attention has shifted to post-translational modifications (PTMs), particularly phosphorylation and ubiquitination, which are crucial for cellular pathways affecting growth, signalling, and protein synthesis.

One of the research focuses is on ER (endoplasmic reticulum) stress mechanisms in CHO cells. This stress can lead to bottlenecks in producing correctly folded proteins, impacting the overall yield of biopharmaceuticals. By inducing artificial ER stress in CHO cells using specific compounds (e.g., thapsigargin and tunicamycin), researchers aim to map and understand the unfolded protein response (UPR) and degradation pathways.

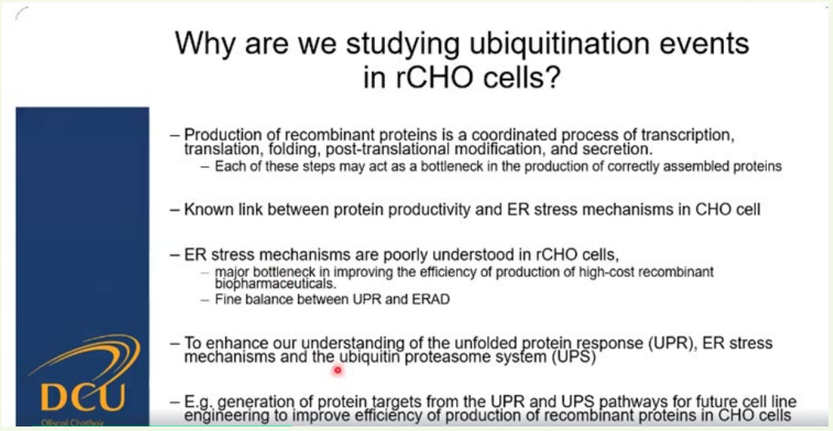
Ubiquitination is a cellular process that tags proteins for degradation. Dr. Meleady's team studied ubiquitination events to understand how ER stress affects protein production and folding in CHO cells. They used mass spectrometry to identify these ubiquitination sites, offering insights into cellular pathways impacted by stress.

The team enriches and analyses ubiquitinated peptides using immunoprecipitation and Liquid Chromatography-Mass Spectrometry (LC-MS). By introducing a proteasomal inhibitor (MG-132), they prevent the degradation of ubiquitinated proteins. This allows for an in-depth analysis of these cellular modifications.

The research identified approximately 5,000 ubiquitinated peptides in CHO cells, enhancing the understanding of ER stress mechanisms. By combining ER stress inducers and proteasomal inhibitors, the team has observed a significant enrichment in ubiquitinated peptides, indicating the potential for identifying novel targets for engineering CHO cells.

Improving the understanding of CHO cells' stress response and protein degradation pathways can lead to the development of more stress-resistant cells. This can increase the efficiency of biopharmaceutical production, potentially reducing costs and speeding up the availability of new therapeutics.

Overall, Dr. Meleady's research aims to unravel the complex processes within CHO cells to optimise biotherapeutic production. The focus on proteomics and post-translational modifications such as ubiquitination is something that has not been widely developed yet.



Developing Brain Organoids to Bridge the Gap Between Preclinical Models and Human Therapeutics

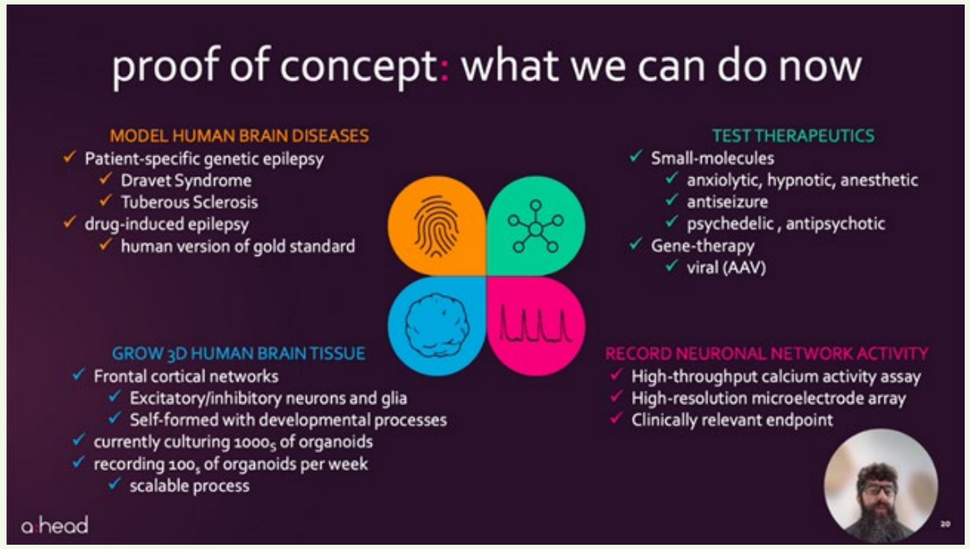
Josh Bagley, Chief Scientific Officer at a:head Bio highlighted how a:head Bio uses brain organoids for drug discovery in central nervous system (CNS) therapeutics. He provided a brief overview of the evolution of CNS therapeutics, highlighting how early CNS drugs were discovered through serendipity and later through an understanding of neurotransmitters. Traditional drug discovery has often used target-centric methods, but recent advances in human stem cell technology now allow researchers to grow human cells in vitro and use them for drug testing.

To bridge the translational gap between preclinical and clinical research within the CNS therapeutic space, Bagley posited that brain organoids offer a solution. Animal models and traditional in vitro studies do not always accurately predict human responses. Whereas, brain organoids, derived from patient cells, offer a more human-relevant, scalable, and accessible model for drug screening. These organoids replicate aspects of human brain tissue, allowing various forms of analyses such as omics, histology, and electrical recordings.

In the drug discovery pipeline, human brain organoids are used to model disease phenotypes and serve as the basis for developing screening assays. These assays can then be used for functional genomics and preclinical development, potentially leading to more effective clinical trials. Bagley described brain organoids as possessing complex layered structures and functional neuronal networks similar to the human brain. These characteristics make them suitable for studying diseases and testing therapeutic interventions.

Bagley outlined the various ways organoids can be used in research, including high-throughput screening for drugs, modelling genetic diseases like Dravet Syndrome, and assessing drug effects on network activity. He also mentioned that using multi-electrode arrays to record electrical activity offers insights into brain network dynamics and connectivity. These are critical for studying neurological and psychiatric disorders.

In conclusion, Bagley stressed the potential of brain organoids for modelling human brain diseases, testing small molecules, gene therapies, and ultimately for CNS drug discovery. a:head Bio aims to expand their organoid models beyond epilepsy to create a scalable platform for higher throughput drug screening and develop new therapeutics for CNS diseases.



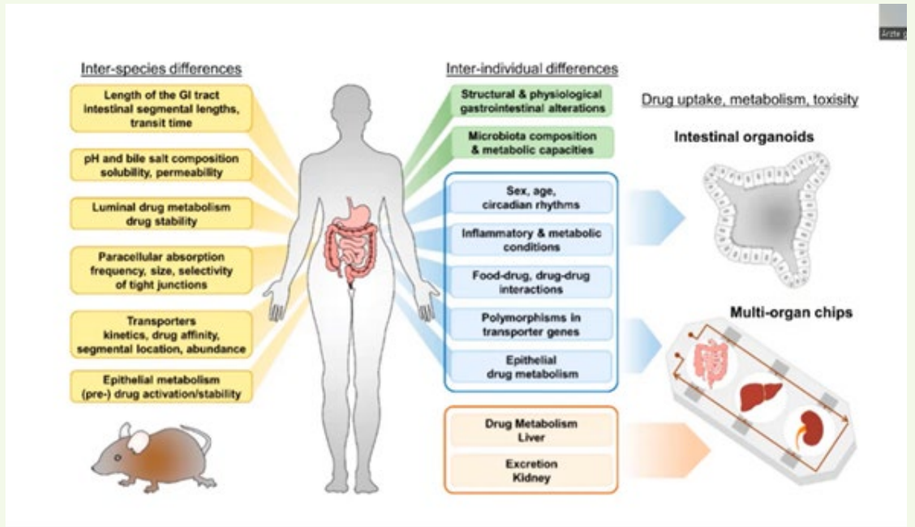
Leveraging Intestinal Organoids to Overcome Challenges in Drug Development

Tamara Zietek, Chief Executive Officer, Doctors Against Animal Experiments examined the limitations of current animal models in drug development and the potential of human-based models like intestinal organoids. She gave an outline of clinical success rates and explained that despite intensive animal testing clinical success rate of new drugs remains very low, averaging only 6.7% in recent years. Zietek stated that this low success rate has even decreased over the last 20 years, with failure rates averaging approximately 92%.

Zietek conducted a scoping review to analyse and verify the last 60 years of drug research. The analysis showed that 76% of these drug failures are due to biological factors, often because animal testing does not accurately predict human outcomes. The differences in biology between species and even among humans themselves (e.g., gender, age, individual response to treatment) make animal models unreliable.

She stressed the need for more human-based model systems, introducing advanced in vitro technologies like 3D organoids, which are complex, multicellular models derived from human cells that mimic the function of real organs. Zietek discussed her work at the Technical University of Munich on intestinal organoids, which they used to study drug uptake, metabolism, and toxicity. Her team focused on enterocytes and enteroendocrine cells within the organoids and demonstrated their ability to express transporters and hormones like GLP-1, crucial for drug testing.

The presentation highlighted that organoids display differences in drug uptake between species. For example, drugs transported in mouse organoids were sometimes not transported in human organoids, reiterating that animal models cannot reliably predict human outcomes. Additionally, the team developed methods to measure intracellular processes in organoids, such as transport activities and proton concentration changes, which are important for understanding drug mechanisms.



Zietek concluded by pointing to the significant advancements in non-animal technologies over the past decade, noting that these models now exist for nearly every human organ. She introduced the NAT (Non-Animal Technologies) database, an online resource with over 2,000 entries on various human-based models, aimed at aiding researchers in finding alternatives to animal testing. She encouraged the audience to use and contribute to this database, promoting a shift towards more effective, human-relevant drug testing methods.

Report Conclusion

This ebook explored the significant advancements made in 3D cell models and the importance of bioprocessing and automation technologies in driving progress in cell culture. While there are still challenges in optimising cell culture and bioprocessing, these industry experts and academics have shown how best practices such as automation, inter-disciplinary collaboration, sourcing quality materials, and establishing standardised regulatory frameworks are key to advancing cell culture. Scaling up 3D cell culture presents a challenge for the future and advanced cell culture models are yet to realise their full potential.

However, there have been important strides toward developing validation strategies and making these advanced cell culture applications available for clinical development. Insights from experts in this ebook estimated that this would be achievable within the next 2-3 years.

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