



Animal Symposia and Workshops

A-1

Fitting Organoids into the Spectrum of Available 3D Culture Models. TERRY RISS. Promega Corporation, 2800 Woods Hollow Road, Madison, WI 53711-5399. Email: terry.riss@promega.com

Among the goals for using 3D culture systems are to develop models that are more predictive of in vivo biology compared to traditional monolayers of cells grown on plastic. The use of 3D cell culture models is growing to include a broad spectrum of approaches. The simplistic end of the spectrum is represented by tumor cell spheroids formed and assayed in the same well using automated homogeneous protocols for high throughput screening. The complex end of the spectrum of in vitro models is represented by microphysiological systems (MPS) composed of multiple stem cell derived organoids interconnected by microfluidic channels of medium representing the in vivo vascular connection of organs. The middle of the spectrum is represented by numerous models using stem cell-derived organoids. A challenge for researchers is to choose a “fit-for-purpose” model considering the compromise between complexity and cost. Assay developers should choose a model that is as simple as possible, but as complex as needed to answer the experimental question. A variety of stem cell-derived organoid models are being developed to fulfill the demand for physiologically relevant model systems.

A-2

Introducing a ‘Phase 0’ in Clinical Trials with Precise Organoid-based Disease Models. COURTNEY TINDLE. HUMANOID Center of Research Excellence (CoRE)TM, University of California, San Diego, 9500 Gilman Drive, George Palade Laboratory, Lab Rm 238 E, San Diego, CA, 92093. Email: ctindle@health.ucsd.edu

Because ‘*mice are not men*’, murine models of chronic human diseases rarely recapitulate the complexity of the disease in humans, which has been implicated in failed

translation of many discoveries. Since their invention, human stem-cell derived organoids have emerged as the flagship model to bridge this translational gap. To improve precision in disease modeling we have successfully added 3 new aspects: (i) Optimized the use of adult stem cell derived organoids from diseased tissues which reflect not just the genetics, but the epigenetic aspects of the diseased cell states; (ii) Incorporated them in co-culture studies with pathogens and other cell types to increase complexity of the diseased tissues; (iii) Rigorous vetting of the engineered models using precise computational tools (i.e., gene signatures derived from disease maps). We have successfully implemented these in Inflammatory bowel disease (IBD, Ulcerative colitis and Crohn’s Disease), COVID-19 lung, organ fibrosis, and GI cancers. Once built and validated, these disease models enable the discovery of novel biology and enable the validation of biomarkers and therapeutic targets in ‘Phase 0’ trials. This ‘Phase 0’ approach is designed to improve both precision and personalization, and in doing so, close the translational gap in modern medicine.

A-3

Initiation, Expansion, and Cryopreservation of Patient-derived Organoids from the Human Cancer Models Initiative. JAMES CLINTON. American Type Culture Collection, 217 Perry Parkway, Gaithersburg, MD 20877. Email: jclinton@atcc.org

ATCC has partnered with the National Cancer Institute to support the Human Cancer Models Initiative, an international collaborative effort to generate hundreds of novel human in vitro cancer models, including three-dimensional patient-derived organoids. These models are supported with clinical and molecular annotation to study cancer, identify and target novel therapies, and facilitate translational cancer research. To date ATCC has authenticated, expanded, preserved, and made available to the research community over 100 patient-derived cancer organoids from a variety of primary tissue sites including colon, stomach, pancreas,

lung, mammary and esophagus. Here we provide an overview of the HCMI, and an introduction to the use, expansion, and cryopreservation of cancer organoids from a practical, hands-on laboratory perspective.

A-4

Brain Organoid Technology: A Versatile Tool to Study the Human Brain. PINAR MESCI. University of California, San Diego, Sanford Consortium for Regenerative Medicine - Muotri Lab, 2880 Torrey Pines Scenic Drive, Room 3108, La Jolla, CA 92093-0695. Email: pmesci@ucsd.edu

Brain organoids mimic the developing human brain both at the cellular and functional levels. The brain organoid technology offers an untapped potential to study the development of the human brain but also to investigate a wide range of neurological disorders. Here, we will present an overview of the use of this technology in a wide range of applications including the modeling of Zika virus and SARS-CoV-2 infections, glioblastomas, autism and the study of the impact of microgravity.

A-5

Beyond Cultrex BME: Ultimatrix and Other Matrices for Stem Cells and Organoids. KEVIN C. FLYNN, Maria Sol Degese, Xi Lu, and Marnelle Andersen. Stem Cell & Gene Therapy, Bio-Techne, 614 McKinley Place NE, Minneapolis, MN 55413. Email: kevin.flynn@bio-techne.com

Stem Cells, 3D cell culture and organoids have become powerful models for emulating *in vivo* physiology. While Basement Membrane Extracts (BMEs) like Cultrex, Geltrex and Matrigel have been widely used for decades, we have developed a novel manufacturing process to improve the consistency, performance and versatility of this matrix. Here I will review the characteristics of Ultimatrix and how these translate into improved functionality which is tunable across various 3D and 2D cell culture models. In addition, I will discuss more targeted protein engineering approaches to improve the function of ECM proteins such as vitronectin for defined stem cell cultivation.

A-6

Dentin Extracellular Matrix: Biological and Mechanical Properties for Bioinspired Tissue Engineering. M. R. CARILHO, Northwestern University, 555 31st St., Science Hall 211-X, Downers Grove, IL 60515. mrocha@northwestern.edu

Knowledge of the structural organization, microarchitecture and mechanical properties of dentin has expanded substantially during the past two decades. The existing literature

indicates that both the inorganic and organic structural components of the dentin extracellular matrix play a critical role in various mechanisms that influence tissue properties. Moreover, insights into the bioactivity of dentin extracellular matrix and its bound biomolecules have drawn great interest towards the exploitation of this substrate for tissue bioengineering applications. In this session, the structural and mechanical features of the extracellular matrix of human dentin, with its intricate three-dimensional complexity, will be comprehensively discussed to provide a better understanding on its potential to serve as a scaffold for future application in regenerative dentistry.

A-7

From Assay to Products – Lessons Learned. THOMAS HARTUNG. Johns Hopkins Bloomberg School of Public Health, 615 N. Wolfe St., W7032, Baltimore, MD 21205. Email: thartun1@jhu.edu

From the ivory tower of academia to success in the biotech and biomedical market is a difficult path. Too often, test developers have rather naïve ideas about market needs and paths to successful products. From the experiences of an academic technology transfer center, the European Commission's validation body for alternative methods to entrepreneurial experiences with academic spin-offs, this talk considers test readiness criteria, standardization and benchmarking, the role of IPR and validation as well as expectation management. The double-edged sword of commercialization as well as the (missed) opportunities of market forces are discussed. The author draws on experiences and examples from the safety sciences over three decades.

A-8

From Lab to Product: Commercialization Paths for Medical Diagnostic Tests. HADLEY SIKES. Massachusetts Institute of Technology, Department of Chemical Engineering, 77 Massachusetts Avenue, Room 66-350, Cambridge, MA 02139. Email: sikes@mit.edu

The COVID-19 pandemic spurred investment in a wide variety of technologies that had not previously been commercialized or approved for use in medical diagnostic testing. This talk will share a case study in rapidly incorporating one such technology into two types of tests, one that detects the virus and one that detects a person's immune response to either vaccination or infection. The steps and hurdles involved in progressing from peer-reviewed publications and intellectual property filings to a new product will be presented. Three distinct paths to commercialization will be discussed, as will the regulatory processes in two different countries.

A-9

From Academia to Industry – the Importance of Good Laboratory Practices Guidelines for Developing Consistent Study Execution. JOHN HARBELL. JHarbell Consulting LLC, 16334 Sunset Valley Drive, Dallas, TX 75248. Email: johnharbell@sbcglobal.net

The ability to reproduce experimental models and results is a cornerstone of the scientific method. When properly done, *in vitro* research holds the promise of highly reproducible and reliable findings. Much of this promise is found in two elements of *in vitro* research. One, *in vitro* testing methods and models offer a high degree of control over the testing system, exposure conditions, consistency, and endpoint measures. Two, *in vitro* researchers possess the ability to design and perform the appropriate controls for each assay to verify the integrity of the testing system and proper assay execution. Not surprisingly, achieving this cornerstone is critical if the researcher seeks to use these data to bring a “product” to the market with regulatory agency approval. *In vitro* methods and models are increasingly a part of the regulatory package submitted in support of drug development, chemical safety, and pesticide registration. This presentation will address the larger aspects of assay execution and uniformity, maintenance of consistency over time, endpoint measures, and the importance of the concurrent positive control incorporation into the study method. Discussed examples will include the importance of training, instrument calibration and reagent controls, all of which are important for the development of testing models in both academic and industrial settings. Moreover, this presentation puts to rest any concern that adherence to test system design and precision assay execution is an undue burden. Further, it suggests that Good Laboratory Practices (GLP) Guidelines provide many good lessons about consistency of process and precision in execution.

A-12

Image Cytometry–based Method Used to Measure Cellular and Subcellular Biology for High-content Screening. NICHOLAS RADIO. Thermo Fisher Scientific. Email: nicholas.radio@thermofisher.com

High-Content Screening (HCS) is an image cytometry–based method used to measure cellular and subcellular biologies amenable to screening in the drug discovery space. The automated nature of both imaging and quantitative analysis enables the throughput ability to measure multiple time points and pharmacological concentrations that are both time- and resource- prohibitive to accomplish using conventional, manual methods. This technical seminar will focus on

best practices to accelerate drug discovery using HCS technology. Practical considerations will be reviewed, including statistical methods to help prioritize which markers are chosen from assay development before scaling to screening campaigns. We will also investigate new technological advancements that can further increase the throughput required for high-throughput screening considerations.

A-13

Application of Single-Cell Technologies in Biomedical Research. J. T. CHANG. Department of Medicine, University of California San Diego, La Jolla, CA. Email: changj@ucsd.edu

Emerging single-cell technologies have recently been used extensively to probe the dynamic protein, transcriptomic, and epigenetic patterns within a wide range of immune cell types in health and disease. Inflammatory bowel disease (IBD) encompasses a spectrum of gastrointestinal disorders driven by dysregulated immune responses against gut microbiota. We integrated single-cell RNA and antigen receptor sequencing to elucidate key components, cellular states, and clonal relationships of the peripheral and gastrointestinal mucosal immune systems in health and ulcerative colitis (UC). We observed heterogeneity in CD8⁺ tissue-resident memory (T_{RM}) cells in colonic tissue, with four transcriptionally distinct states of differentiation observed across health and disease. In the setting of UC, there was a marked shift of clonally related CD8⁺ T_{RM} cells toward an inflammatory state, mediated, in part, by increased expression of the T-box transcription factor Eomesodermin. Together, these results suggest a role for CD8⁺ T_{RM} cells in IBD.

A-14

Defining Epithelial Development at the Single Cell Level. S. X. ATWOOD, S. Wang, M. L. Drummond, and A. S. Stabell. University of California Irvine, Developmental and Cell Biology, 4340 McGaugh Hall, Irvine, CA 92697. Email: satwood@uci.edu

How stem cells give rise to epidermis is unclear despite the crucial role the epidermis plays in barrier and appendage formation. Here we use single cell RNA-sequencing to interrogate basal stem cell heterogeneity of human interfollicular epidermis and find four spatially distinct stem cell populations at the top and bottom of rete ridges and transitional positions between the basal and suprabasal epidermal layers. Alterations in differentially expressed transitional basal stem cell genes result in severe thinning of human skin equivalent organoids, validating their essential role in epidermal homeostasis and reinforcing the critical nature of basal stem cell heterogeneity.

A-15

Utilizing Single Cell Analyses to Characterize Models of Human Cortical Organoids. APARNA BHADURI. Department of Biological Chemistry, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA 90095. Email: ABhaduri@mednet.ucla.edu

The human cortex is the outermost layer of the brain that enables complex cognition, judgment and perception. Each of the areas of the cortex contribute to unique functionality, and begin emerging during developmental timepoints. Using single-cell RNA-sequencing, we have created an atlas of cell types that exist during normal human cortical development across peak stages of neurogenesis. These data identify unique cell types, trajectories and maturation

signatures across developmental time and between cortical areas. Cortical organoids are stem cell derived models of the developing human brain. When comparing these features of normal human development to single-cell data of cortical organoids, we identify many similarities as well as some important differences. Namely, we identify that cell subtype specification is less precise in cortical organoids and that this correlates to upregulated metabolic stress. Additionally, although area-specific cell populations can be made, they are not structurally organized in the organoid as they are in the primary human brain. Ongoing work in the lab is seeking to better understand how metabolism and transcriptional inputs drive these features of organoid development and how they can be studied in cortical organoids to elucidate key principles of human cortical development.