8th International Conference on Stem Cell Engineering

October 7-9, 2022 | Boston Marriott Cambridge | Boston, MA

ORGANIZED BY SOCIETY FOR BIOLOGICAL ENGINEERING

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TIPS FOR A SUCCESSFUL MEETING

Say **hello** to everyone.
You might make someone's day.

Introduce yourself to people you don't know.
They may be your next good friends.

Stop and **smile**.
You will brighten the room considerably.

Be **understanding**.
Everybody makes mistakes.

Help those with less experience.
We were all novices at some point.

Respect others.
We all have something valuable to contribute.

Value staff and volunteers.
They are here for you.

Be **kind**.
You will never like everybody, but you can be cordial to all.

Enjoy the meeting!
You can have fun while sharing,learning and networking.

Abstracts appear as submitted by their authors. Neither the American Institute of Chemical Engineers (AIChE) and its entities, nor the employers affiliated with the authors or presenting speakers, are responsible for the content of the abstracts.

Greetings

Welcome Address

After a challenging year, we are certainly happy to officially welcome you to the 8th Conference on Stem Cell Engineering brought to you by the Society for Biological Engineers (SBE), an American Institute for Chemical Engineers Technological Community. We are very happy with the interactive and inspiring technical program planned for this in-person conference.

The Stem Cell Engineering Conference is a world-leading conference to share premium developments and achievements in the field. This conference will bring together leaders and trainees from cutting edge fields of stem cell engineering to develop the next-generation of stem cell technologies. We hope that you will enjoy hearing about the state-of-the-art science and technology in stem cell engineering that will be shared at the conference.

Our program this year will provide meeting attendees with compelling technical sessions, inspiring lectures, and valuable opportunities to network with speakers, poster presenters, exhibitors, and colleagues.. We thank the many contributions of our Organizing Committee and session chairs for their invaluable assistance in helping to shape our program.

Finally, we would like to thank you for attending the conference. We hope that these next three days will be inspiring, productive, stimulating, and enjoyable.

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Mo Ebrahimkhani, *University of Pittsburgh, USA – Conference Chair*

Omer Yilmaz, *Massachusetts Institute of Technology, USA - Conference Chair*

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Jianping Fu, *University of Michigan, Ann Arbor, USA*

Nuria Montserrat, *Institute for Bioengineering of Catalonia (IBEC), Spain*

Linda Griffith, *Massachusetts Institute of Technology, USA*

Jason Spence, *University of Michigan Medical School, Ann Arbor, USA*

Alex Shalek, *Massachusetts Institute of*

Technology, USA

Organized by the Society for Biological Engineering

Technical Program

Day 1 Oct 7, 2022		
Start Time	**End Time**	
8:00 AM	**8:50 AM**	**Registration**
8:50 AM	**9:00 AM**	**Welcome Remarks** **Mo Ebrahimkhani, University of Pittsburgh** **Omer Yilmaz, Massachusetts Institute of Technology**
9:00 AM	**9:45 AM**	**Keynote Presentation: Linda Griffith,** **Massachusetts Institute of Technology**
9:45 AM	**10:30 AM**	**Coffee Break**
10:30 AM	**12:00 AM**	**Exploring Physiology, the Microbiome and disease with organoids (I)**
10:30 AM	11:00 AM	Nuria Montserrat, Institute for Bioengineering of Catalonia
11:00 AM	11:30 AM	Kara Mckinley, Harvard University *"Regeneration in the uterus"*
11:30 AM	11:45 AM	George Eng, Massachusetts Institute of Technology
11:45 AM	**1:30 PM**	**Lunch Break**
1:30 PM	**3:00 PM**	**Exploring Physiology, the Microbiome and disease with organoids (II)**
1:30 PM	2:00 PM	Carla Kim, Boston Children's Hospital *"Organoid assays for in vitro and in vivo models of lung disease & cancer"*
2:00 PM	2:30 PM	Semir Beyaz, Cold Spring Harbor Laboratory
2:30 PM	2:45 PM	Xiaoping Bao, Purdue University *"Engineer CAR-Neutrophils from Human Pluripotent Stem Cells for Targeted Chemoimmunotherapy"*
2:45 PM	**3:30 PM**	**Coffee Break**
3:30 PM	**5:00 PM**	**Systems Biology and Single Cell Dynamics**
3:30 PM	4:00 PM	Alex Shalek, Massachusetts Institute of Technology *"Does cell state matter in cancer?"*
4:00 PM	4:30 PM	Pamela Hoodless, British Columbia Cancer Research Institute *"Defining developmental pathways during liver specification using single cell analysis"*
4:30 PM	5:00 PM	Robert Schwartz, Cornell University *"Development of tissue-engineered liver-like tissue"*
5:00 PM	5:15 PM	Omid Mashinchian *"An Engineered Multicellular Stem Cell Niche to Study Skeletal Muscle Physiology and Function"*

Technical Program

Day 2 October 8, 2022		
Start Time	**End Time**	
9:30 AM	**10:00 AM**	**Registration**
10:00 AM	**1:00 PM**	**Synthetic Biology: Programming Fate and Organization**
10:00 AM	10:30 AM	Nika Shakiba, University of British Columbia *"Competition in cell fate programming and development"*
10:30 AM	11:00 AM	Leonardo Morsut, University of Southern California *"Learning to Program Tissue Development with Artificial Genetic Circuits"*
11:00 AM	**11:30 AM**	**Coffee Break**
11:30 AM	12:00 PM	Giorgia Quadrato, University of Southern California *"Upgrading the physiological relevance of human brain organoids"*
12:00 PM	12:30 PM	Katie E. Galloway, Massachusetts Institute of Technology
12:30 PM	12:45 PM	Alex Ng, GC Therapeutics
12:45 PM	1:00 PM	Xiaojun Lance Lian, Pennsylvania State University *"Modrna-Based CRISPR Systems for Robust Genome Editing in Human Pluripotent Stem Cells"*
1:00 PM	**2:15 PM**	**Lunch Break**
2:15 PM	**4:55 PM**	**Synthetic Developmental Biology & Early Developmental Engineering**
2:15 PM	2:45 PM	Ben Hurlbut, Arizona State University
2:45 PM	3:15 PM	Jianping Fu, University of Michigan *"Synthetic Embryology for Constructing Human Embryo and Organ Models"*
3:15 PM	**3:35 PM**	**Coffee Break**
3:35 PM	4:05 PM	Berna Sozen, Yale University *"Design principles of multicellular organisation and patterning in embryonic development"*
4:05 PM	4:35 PM	Mo Ebrahimkhani, University of Pittsburgh
4:35 PM	4:55 PM	Coffee Break
4:55 PM	**5:40 PM**	**Keynote Presentation: Ali Brivanlou, The Rockefeller University**
5:40 PM	**7:00 PM**	**Poster and Reception**

Technical Program

Day 3 October 9, 2022		
Start Time	**End Time**	
9:00 AM	**9:45 AM**	**Keynote Presentation: Joshua Leonard, Northwestern University**
9:45 AM	**10:15 AM**	**Coffee Break**
10:15 AM	**11:45 AM**	**Tools and Technologies**
10:15 AM	10:45 AM	Daniel Shiwarski, Carnegie Mellon University
10:45 AM	11:15 AM	Mehdi Jorfi, Harvard University *"Dissecting the complexities of Alzheimer's disease with multicellular models of the human brain"*
11:15 AM	11:45 AM	Sahand Hormoz, Harvard University *"Reconstructing the lineage trees of individual stem cells"*
11:45 AM	**12:00 PM**	**Closing Remarks** **Mo Ebrahimkhani, University of Pittsburgh** **Omer Yilmaz, Massachusetts Institute of Technology**

Speaker Biographies

Linda G. Griffith

MIT

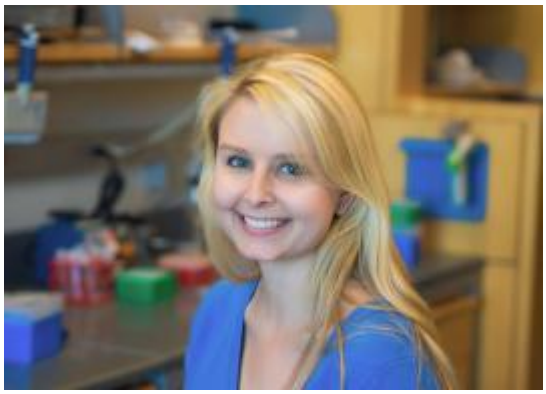
Linda G. Griffith, PhD, is the School of Engineering Teaching Innovation Professor of Biological and Mechanical Engineering at MIT, where she directs the Center for Gynepathology Research and “Bio-Mimetics”, a DARPA/NIH-funded Microphysiological Systems Program. Dr. Griffith received a Bachelor's Degree from Georgia Tech and a PhD degree from the University of California at Berkeley, both in chemical engineering. Dr. Griffith’s research is in the field of regenerative medicine and tissue engineering. Her laboratory, in collaboration with J. Upton and C. Vacanti, was the first to combine a degradable scaffold with donor cells to create tissue-engineered cartilage in the shape of a human ear. The 3D Printing Process she co-invented for creation of complex scaffolds is used for manufacture of FDA-approved scaffolds for bone regeneration. She is also a pioneer in devising ways to control nano-scale stimulation of cells by molecular cues, and in creation of 3D tissue models for drug development. Her work has been featured on television documentary shows including Scientific American Frontiers. She is a member of the National Academy of Engineering and the recipient of a MacArthur Foundation Fellowship “genius grant”, the Popular Science Brilliant 10 Award, NSF Presidential Young Investigator Award, the MIT Class of 1960 Teaching Innovation Award, Radcliffe Fellow and several awards from professional societies. She has served as a member of the Advisory Councils for the National Institute for Dental and Craniofacial Research and the National Institute of Arthritis, Musculoskeletal and Skin Diseases at NIH. As chair of the Undergraduate Curriculum Committee for Biological Engineering at MIT, she led development of the new Biological Engineering SB degree program, which was approved in 2005 as MIT’s first new undergraduate major in 39 years.

Josh Leonard

Northwestern University

Dr. Josh Leonard is the Associate Professor of Chemical & Biological Engineering at Northwestern University. He earned his Ph. D. in Chemical Engineering from University of California, Berkeley in 2006. He is currently the Principal Investigator of the Laboratory for Cellular Devices and Biomolecular Engineering at Northwestern University, where the Leonard Lab works at the interface of systems biology and synthetic biology in order to probe and program the function of complex, multicellular systems to develop transformative biotechnologies and enable a new paradigm of design-driven medicine. Using the tools of synthetic biology, biomolecular engineering, computational systems biology, and gene therapy, the Leonard Lab develops technologies including programmable cell-based “devices,” immune therapies for cancer and chronic disease, smart vaccines, biosensors for global health applications, and tools for advanced metabolic engineering. By bringing an engineering approach to the investigation, design, and construction of biological systems, the Leonard group is advancing the frontiers of design-driven medicine to address unmet medical needs and create safe, effective, and long-lasting treatment options that improve both quantity and quality of life.

Speaker Biographies



Kara McKinley
Harvard University
Kara McKinley is an Assistant Professor in the Department of Stem Cell and Regenerative Biology at Harvard University and principal faculty of the Harvard Stem Cell Institute. Her lab studies how the uterine lining (endometrium) regenerates after menstruation with the goal of improving care for people with endometrial pathologies and/or menstrual experiences that interfere with their quality of life. She received her Ph.D. from MIT in 2016 under the mentorship of Iain Cheeseman. She performed postdoctoral training in the laboratories of Ron Vale and Ophir Klein at the University of California, San Francisco. She is also the founder of Leading Edge, an initiative to improve the gender diversity of faculty in the biomedical sciences.

Giorgia Quadrato
University of Southern California

Giorgia Quadrato is an assistant professor in the Broad CIRM Center and Department of Stem Cell Biology and Regenerative Medicine at the University of Southern California (USC). She previously worked as a research associate in the Arlotta Laboratory at Harvard University and the Broad Institute of Massachusetts Institute of Technology and Harvard University. Dr. Quadrato's lab focuses on understanding the cellular and molecular basis of human brain development and disease. By combining the use of emerging models of the human brain with single cell omics approaches, she is aiming to identify cell type specific disease mechanisms, and above all, new treatments for human neurodevelopmental disorders. Dr. Quadrato earned her BS, MS, from the University of Milano-Bicocca (Italy) and her PhD in pharmacogenomic biotechnology from the University of Novara (Italy)

Berna Sozen
Yale University

Berna Sozen is Assistant Professor at the Department of Genetics, Yale School of Medicine and affiliated faculty of Yale Stem Cell Center. Dr. Sozen first trained as a Reproductive Biologist at Akdeniz University, Turkey and later Developmental Stem Cell Biologist at the University of Cambridge, UK. She then undertook her post-doctoral research first at Cambridge-UK, and later at the Caltech-USA during which she developed in vitro systems to model mammalian early embryogenesis using various types of embryo-derived stem cells, in Magdalena Zernicka-Goetz group. Her independent group is now studying how embryo-environment interactions direct developmental patterning in human embryogenesis with long-term aim to understand origins of developmental disease.

Speaker Biographies

Ben Hurlbut
Arizona State University

J. Benjamin Hurlbut is trained in the history of modern biomedical and life sciences. His research lies at the intersection of science and technology studies, bioethics and political theory. He studies the changing relationships between science, politics and law in the governance of biomedical research and innovation in the 20th and 21st centuries.

Focusing on controversy around morally and technically complex problems in areas such as human embryonic stem cell research and genomics, Hurlbut examines the interplay of science and technology with shifting notions of democracy, of religious and moral pluralism, and of public reason.

He is author of Experiments in Democracy: Human Embryo Research and the Politics of Bioethics (Columbia University Press, 2017). He holds an A.B. from Stanford University, and a Ph.D. in the History of Science from Harvard University. He was a postdoctoral fellow in the Program on Science, Technology, and Society at Harvard Kennedy School.

Katie Galloway
MIT

Katie Galloway is the Charles and Hilda Roddey Career Development Professor in Chemical Engineering at MIT. Katie earned her BS in Chemical Engineering from UC Berkeley, PhD in Chemical Engineering at Caltech, and did her postdoc at USC Stem Cell before starting at MIT in the fall of 2019. As a chemical engineer working in molecular systems biology, her research focuses on elucidating the fundamental principles of constructing and integrating synthetic circuitry to drive cellular behaviors. Her team leverages synthetic biology to transform how we understand cellular transitions and engineer cellular therapies. Her research has been featured in Science, Cell Stem Cell, Cell Systems, and Development. She has won multiple fellowships and awards including the NIH Maximizing Investigators' Research Award (MIRA) (R35), NIH F32, and Caltech's Everhart Award.

Mehdi Jorfi
Harvard Medical School

Mehdi Jorfi, Ph.D., is a faculty member in neurology at the Genetics and Aging Research Unit at Massachusetts General Hospital and Harvard Medical School with a joint appointment at the Center for Engineering in Medicine & Surgery. He is currently interested in integrating neurobiology and engineering disciplines to address high-impact problems in neuroscience and medicine. Dr. Jorfi's work takes a multipronged approach to model Alzheimer's human brain on a chip and studies the role of the peripheral immune cells in neurodegenerative diseases. His research utilizes bioengineering, pluripotent stem cell technology, genetic engineering, 3D brain models, and human blood.

He received his Ph.D. in bioengineering from the University of Fribourg, Switzerland, where he worked on developing mechanically adaptive intracortical microelectrodes to understand the multifaceted role of neuroinflammation in disrupting both biologic and abiologic components of the neural interface circuit. He then undertook postdoctoral

research at MIT, focused on organs-on-chips technology, and later at the Massachusetts General Hospital and Harvard Medical School. At Harvard, he continued his research in modeling human brain tissues in microphysiologically relevant 'chips' using stem cell-derived 3D models for studying Alzheimer's disease. Dr. Jorfi has received several awards, including the honorary Faculty Science Prize, HBSI Young Scientist Award, and Mass General Scientific Advisory Committee Distinction Award.

Núria Montserrat Pulido
Institute for Bioengineering of Catalonia

Dr. Nuria Montserrat became interested in organ regeneration and stem cells during her master and PhD training that finished in 2006. The same year she got a Postdoctoral fellowship from the Fundaçao para a Ciência e Tecnología (Portugal). In 2007 she was hired as a post-doctoral researcher at the Hospital of Santa Creu i Sant Pau in Barcelona.

In 2008 she moved to the Center of Regenerative Medicine of Barcelona (CMRB) as a research associate. There, Dr. Montserrat participated in developing strategies for the generation and banking of new induced pluripotent stem cells (iPSCs).

In 2010 she first co-authored how to reprogram cord blood stem cells for the first time (Nature Protocols, 2010). Then she reasoned that iPSCs could be obtained by means of safe strategies with new factors. The work resulted in a high-impact publication in Cell Stem Cell (2013), in where she was the first co-author. She also collaborated in other projects aimed to characterize the genomic integrity of human iPSCs (Nature 2012) as well as in the differentiation of iPSCs towards different lineages (Stem Cells 2011; Nature 2012; Nature Methods 2012, Nature Cell Biology 2013, Nature Communications 2014). Dr. Montserrat has also participated in the generation of platforms for the study of disease progression by means of iPSCs (Nature 2012, Nature Communications 2014).

More recently, she has first co-authored how the reactivation of endogenous pathways can be artificially reactivated and promote heart regeneration in mammals (Cell Stem Cell, 2014). Her expertise in the fields of somatic reprogramming and organ regeneration helped her to develop a massive project was been selected for funding from the European Research Council within the ERC Starting Grant call of 2014.

Semir Beyaz
Cold Spring Harbor Laboratory

Semir Beyaz received his B.S. degree in Molecular Biology and Genetics at Izmir Institute of Technology, where he was ranked 1st in the Faculty of Science in 2009. After working at the Bone Marrow Transplantation Center at Massachusetts General Hospital between 2009-2010, he joined Harvard University, Immunology PhD program. There, he worked in Stuart Orkin's lab at Boston Children's Hospital on the epigenetic regulation of blood cell development and also in Omer Yilmaz's lab at the Koch Institute of Massachusetts Institute of Technology on dietary regulation of intestinal stem cell function and tumorigenesis. After receiving his PhD in 2017, he established his research laboratory at Cold Spring Harbor Laboratory (CSHL) in New York, where he is a CSHL Fellow and Donaldson Trust Translational Fellow. His lab is focusing on how dietary and metabolic perturbations affect the immune system and contribute to the risk of diseases that are associated with immune dysfunction such as cancer. He received several awards for his scientific accomplishments including the Jeffrey Modell Prize, American Association of Immunologists Thermo-Fisher Trainee Achievement Award and TASSA Aziz Sancar Young Scholar Award.

Speaker Biographies

Alex K. Shalek
MIT

Alex K. Shalek is currently the Pfizer-Laubach Career Development Associate Professor at MIT, as well as a Core Member of the Institute for Medical Engineering and Science (IMES), an Associate Professor of Chemistry, and an Extramural Member of The Koch Institute for Integrative Cancer Research. He is also an Institute Member of the Broad Institute, an Associate Member of the Ragon Institute, an Assistant in Immunology at MGH, and an Instructor in Health Sciences and Technology at HMS. His research is directed towards the creation and implementation of new approaches to elucidate cellular and molecular features that inform tissue-level function and dysfunction across the spectrum of human health and disease. Dr. Shalek received his bachelor's degree summa cum laude from Columbia University and his Ph.D. from Harvard University in chemical physics under the guidance of Hongkun Park, and performed postdoctoral training under Hongkun Park and Aviv Regev (Broad/MIT).

Jianping Fu
University of Michigan, Ann Arbor

Dr. Fu received a B.E. degree (2000) from the University of Science and Technology of China (USTC) and a M.S. degree (2002) from the University of California at Los Angeles (UCLA), both in Mechanical Engineering. He earned his Ph.D. degree in Mechanical Engineering from the Massachusetts Institute of Technology (MIT) in 2007 for thesis completed with Dr. Jongyoon Han. Dr. Fu was an American Heart Association Postdoctoral Fellow in Dr. Christopher S. Chen's group at the University of Pennsylvania from 2007 to 2009. Dr. Fu has been at the University of Michigan in the Department of Mechanical Engineering from 2009 to the present, achieving the rank of Professor of Mechanical Engineering in 2020. Dr. Fu also holds courtesy appointments in the Department of Biomedical Engineering and the Department of Cell and Developmental Biology.

Dr. Fu's research contributions have been recognized by more than 30 institutional, national and international awards and honors, including the National Science Foundation CAREER Award (2012), the Rising Star Award from the Biomedical Engineering Society (2016), the Analytical Chemistry Young Innovator Award from the American Chemical Society (2020), and numerous research awards from the University of Michigan. Dr. Fu is a Fellow of the American Institute for Medical and Biological Engineering (AIMBE), the Royal Society of Chemistry (RSC), and the American Society of Mechanical Engineers (ASME). He is also a Senior Member of the Institute of Electrical and Electronics Engineers (IEEE). Dr. Fu is a member of the International Society for Stem Cell Research (ISSCR) Guidelines Working Group (2019-2021) and a council member of the Biomedical Engineering Society Cellular and Molecular Bioengineering Special Interest Group (BMES CMBE-SIG) (2020-2022). Dr. Fu's current research focuses on mechanobiology, stem cell bioengineering, developmental bioengineering, microfluidics and BioMEMS. Dr. Fu and his co-workers' research on modeling human development using human stem cells has contributed significantly to the emerging field of "Artificial Embryos", which was selected by the MIT Technology Review as "10 Breakthrough Technologies of 2018".

Speaker Biographies

Nika Shakiba
University of British Columbia

Dr. Nika Shakiba is an Assistant Professor in the School of Biomedical Engineering (SBME) at UBC. Her research program is interested in understanding the social lives of stem cells. Her lab applies a combined systems and synthetic biology approach to reverse- and forward-engineer the competitive interactions between cells in developmental systems. Prior to joining SBME, Nika was a Postdoctoral Fellow in the Department of Biological Engineering at the Massachusetts Institute of Technology and completed her PhD in the Institute of Biomaterials and Biomedical Engineering at University of Toronto. Nika is a big believer in outreach and mentorship. Beyond her research and teaching, she is passionate about providing equity in mentorship & advice access through her latest project, Advice to a Scientist.

Leonardo Morsut
University of Southern California

Leonardo Morsut, assistant professor of Stem Cell Biology and Regenerative Medicine at USC, was born and raised in Padova, Italy, with an early fascination for science in general, and for embryology in particular. His scientific background is multifaceted, with degrees in Medical Biotechnologies, Math and Developmental Biology from Padova University (Italy). During his PhD in the Stefano Piccolo lab, he investigated roles of morphogen signaling during early mouse embryogenesis; he also described the YAP/TAZ mechanotransduction pathway in a seminal paper that opened the field of transcriptional interpretation of mechanical cues by cells.

In his postdoc in the laboratory of Wendell Lim at UCSF, he developed synthetic biology tools for multicellular systems. The synNotch receptor that he invented there, in collaboration with Kole Roybal, PhD from the same lab, is a modular tool that can be used to engineer new sensing-and-respond behaviors in cells; it has raised wide interest both in the academic as well as the industry setting.

In his “Tissue Development Engineering lab" at USC he applies the synthetic biology framework to implement developmental programs in stem cells in order to guide their development into user-specified tissue in vitro, with applications in tissue engineering and regenerative medicine problems. Specific areas of interests are: developing vascularization systems for in vitro grown organoids, synthetic biology tools to control electric fields in tissues, engineered cell therapy for neurodeneneration and bone and cartilage defects.

Poster Presentations

Poster Titles

1. Yuan Li. Northeastern University. Effects of Physiological Relevant Bile Salts Combination on Duodenal/Ileum Monolayer: Mucin Secretion and Monolayer Integrity
2. Deepak Mishra. MIT. Genetically Engineered Human Pluripotent Stem Cells Utilizing Multi-Step Automated Differentiation Networks for Development of Three-Dimensional Liver Organoids
3. Lei Wang. MIT. From iPSCs to Hematopoietic Lineage: An Endoribonuclease Mediated Cell-Type-Specific Genetic System to Guide Automated Differentiation
4. Sumin Lee. Tomocube Inc. Label-Free Three-Dimensional Morphological Analysis of Live Organoids Using Low-Coherent Holotomography
5. Paulina Eberts. University of Minnesota - Twin Cities. Modeling Amyloid-Beta Transport in hiPSC-Derived Models of the Blood-Brain Barrier
6. Joshua Hislop. University of Pittsburgh School of Medicine. Synthetic Morphogenesis of Human Bilaminar Disc Via Genetically Encoded Transcriptional Programs
7. Ryan LeGraw. University of Pittsburgh. Gene Regulatory Network Analysis and Engineering Directs Development and Vascularization of Multilineage Human Liver Organoids

Poster Abstracts

1. Bile acids/salts (BAs/BSs) are recognized as a family of stimulators that play essential roles in regulating various functions of physiology within the intestinal tract, like promoting the metabolism of dietary lipids, stimulating mucin secretion, and tuning the commensal bacterial community. However, many of the in vivo results were not able to be reproduced by in vitro models. And most in vitro studies were focused on single BA/BS treatments. Here, we set up a bile salts and phosphatidylcholine complex model (BS/PC) that mimics the human physiological intestinal luminal fluid at fasted state. In this experiment, 8-9 days old duodenal and ileal monolayers were exposed to BS/PC for 24 hours. After the exposure, the permeabilities of monolayers and the mucin concentrations were measured by the Lucifer Yellow assay and the Alcian Blue (AB) assay, respectively. ZO-1 and MUC2 proteins on the monolayer were stained to characterize the abundance of tight junction and mucus. According to the confocal imaged and AB assay data, BS/PC exposure altered the morphology of the duodenal monolayer and increased tight junction and mucus production of duodenal/ileal monolayers. The LY assay data showed BS/PC exposure did not affect monolayer permeability significantly, indicating there's no damage of BS/PC to duodenal and ileal monolayers. The results indicated that the complex bile salts could affect the intestinal mucosa in vitro at a physiologically relevant concentration.

2. Three-dimensional (3D) human stem cell-derived systems allow recapitulation of architecture, composition, and physiology of organs. Organoids may be formed by combining individually differentiated cell populations or via introduction of external cues to human induced pluripotent stem cells (hiPSCs) to mimic stages of human development. However, such approaches may limit intercellular interactions or faithful mimicry of a mature organ's structure. Here, we present an approach to genetically engineer hiPSCs with transcriptional and post-transcriptional regulatory networks to achieve multi-step differentiation in a cell-specific, semi-automated manner to form 3D liver organoids. The system relies on two independently operating networks, the first is small-molecule inducible heterogeneous expression of GATA-binding protein 6 (Gata6) to initiate germ layer formation and is capable of directing formation of 3D vascularized liver organoids. The second network operates post-transcriptionally, coupling miRNA sensing of miR-122a-5p and restriction endoribonucleases (ERNs) to autonomously express protein payloads in a hepatic-lineage cell-conditional manner. These engineered iPSCs were grown for thirty days to form 3D liver organoids and when compared to a single-stage network, exhibited 60% increase in urea production, 36% increase in albumin production, and an order of magnitude increase of Cyp3A4 enzyme function while maintaining vasculature and cell compositional diversity. Collectively, our two-stage genetic network approach enabled selective differentiation, maturation, and conditional payload expression for hepatocyte development. We anticipate systems such as ours utilizing cell-specific miRNA sensors and ERN controlled payloads will allow selective differentiation of germ layers and progenitors as well as cell maturation across many existing organoid models.

3. hiPSCs provide unprecedented opportunities for cell therapies against intractable diseases. To produce therapeutic immune cells, recent advances emphasize the inevitable role of endothelial-to-hematopoietic transition (EHT), but still face a significant challenge in building a genetic program for cell-type-specific differentiation. To tackle this challenge, we built a novel platform for genetic programming, where endoribonucleases from the CRISPR family mediate sensing of endogenous cell-type-specific microRNA (miR) and actuate cell differentiation by transcriptional factor (TF) overexpression. We applied this system to differentiate hiPSC into hematopoietic lineages with an intermediate endothelial state. First, we extensively searched databases to identify endothelial-specific miRs and TFs that are critical for EHT. We designed and built high miR sensors and optimized the circuit elements' stoichiometries by a poly-transfection method and achieved a fold change as high as 63. Then we verified the cell type specificity of this sensor using endothelial and non-endothelial cell lines. We monitored the temporal dynamics of endogenous miR during cell differentiation using a programmed differentiation from hiPSCs to endothelial cells via a 4-day protocol. With these solid validations, we replaced the reporter of the high miR sensor with TFs to guide differentiation to the hematopoietic lineage. Our results showed that the miR sensing and TFs actuating systems can derive significant percentages of CD34+CD45+ hematopoietic-like cells from hiPSCs. Together, this genetic system will provide a novel platform to program cell-type-specific differentiation, which will provide insights into the

universal generation of functional and therapeutic immune cells from unlimited and reprogrammable hiPSC sources.

4. Three-dimensional (3D) visualization and morphological dissection of organoids is challenging due to the thickness and its complex structure. Fluorescence-based imaging methods have been widely used; however, those are not suitable for observing morphological changes of growing organoids without damaging the samples. Holotomography (HT) has emerged as a useful tool for imaging live specimens without additional pre-treatment such as fixation, fluorescence labeling and excitation. HT can achieve long-term 3D observation of live specimens for weeks without phototoxicity. Furthermore, the measured individual cell data can be analyzed to elucidate not only the spatio-temporal 3D dynamics with high spatial resolution, but also the quantitative information of its subcellular compartments such as volume and dry mass. In this study, we applied a recently developed low-coherent holotomography imaging system, HT-X1, which provides label-free 3D visualization of live cells and multicellular specimens. Using the HT-X1, we successfully observed in situ 3D morphological dynamics of patient-derived human lung organoids with Matrigel-embedded condition. The location and its thickness of the mucosal layer inside of which has a lower refractive index (RI) were also detected, since HT enables the volumetric reconstruction of RI which provides quantitative morphology analysis with unprecedented precision and resolution. The other subcellular features such as nuclei, nucleoli, mitochondria, lipid droplets, chromosomes, and multiciliate structures were observed with the high resolution of the HT-X1. Throughout the results, we suggest that low-coherent holotomography has an exclusive capability for phenotypic and quantitative studies of diverse types of organoids and other 3D multicellular live specimens including stem cells.

5. One of the earliest detectable changes in sporadic Alzheimer's disease (AD) is dysfunction of the blood-brain barrier (BBB). A decrease in the ability of the BBB to transport amyloid-beta largely contributes to the accumulation of this peptide in the brain, a hallmark feature of AD. The causes of disrupted amyloid-beta transport across the BBB in AD are not fully understood. Here we used an in vitro model of the BBB composed of brain microvascular endothelial-like cells (iBMECs) differentiated from human induced pluripotent stem cells (hiPSCs) to model amyloid-beta transport. iBMEC expression of key receptors involved in amyloid-beta transcytosis, including the low-density lipoprotein receptor-related protein 1 (LRP1) and the receptor for advanced glycation endproducts (RAGE), was confirmed via immunocytochemistry and western blotting. Experimentation with iBMECs is commonly conducted 2-3 days after initial barrier formation. However, confocal microscopy revealed that proper polarization of the aforementioned receptors required iBMEC barriers to maintain a high transendothelial electrical resistance (TEER) (>1000 Ω·cm2) for at least eight days after barrier formation. Polarized barriers exhibited greater rates of amyloid-beta transport and lower sodium fluorescein permeability compared to non-polarized barriers. To probe the changes observed in polarized versus non-polarized barriers, proteomic profiling was performed using 16-plex TMT-LC/LC-MS/MS. Principal component analysis and hierarchical clustering of these data revealed that polarized and non-polarized barriers have distinct proteomes. This collective work demonstrates the suitability of iBMECs for examining amyloid-beta transport and suggests that length of time in culture is a parameter that should be taken into account when working with iBMECs.

6. Implantation of human embryo commences a critical developmental stage that comprises profound morphogenetic alteration of embryonic and extraembryonic tissues, axis formation and gastrulation events. Our mechanistic knowledge of this window of human life remains limited due to restricted access to natural healthy samples for both technical and ethical reasons. Here we describe a system that employs human induced pluripotent stem cells engineered with an inducible human transcription factor gene circuit to demonstrate genetically guided cell fates with single-cell transcriptional profiles similar to post-implantation embryonic and extraembryonic lineages. We show self-organization of these populations into three-dimensional multifate epiblast-like compartments surrounded by a yolk sac hypoblast layer. We observe self-organization and tissue boundary formation that recapitulates yolk sac-like tissue specification, the formation of bilaminar disc-like structure and the development of an amniotic-like cavity. This approach provides a simple platform for studying peri-implantation embryonic fate decisions and for the exploration of synthetic developmental engineering using a genetically encoded transcriptional program.

7. Pluripotent stem cell (PSC)-derived organoids have emerged as novel multicellular models of human tissue development but display immature phenotypes, aberrant tissue fates, and a limited subset of cells. Here, we demonstrate that integrated analysis and engineering of gene regulatory networks (GRNs) in PSC-derived multilineage human liver organoids direct maturation and vascular morphogenesis in vitro. Overexpression of PROX1 and ATF5, combined with targeted CRISPR-based transcriptional activation of endogenous CYP3A4, reprograms tissue GRNs and improves native liver functions, such as FXR signaling, CYP3A4 enzymatic activity, and stromal cell reactivity. The engineered tissues possess superior liver identity when compared with other PSC-derived liver organoids and show the presence of hepatocyte, biliary, endothelial, and stellate-like cell populations in single-cell RNA-seq analysis. Finally, they show hepatic functions when studied in vivo. Collectively, our approach provides an experimental framework to direct organogenesis in vitro by systematically probing molecular pathways and transcriptional networks that promote tissue development.

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