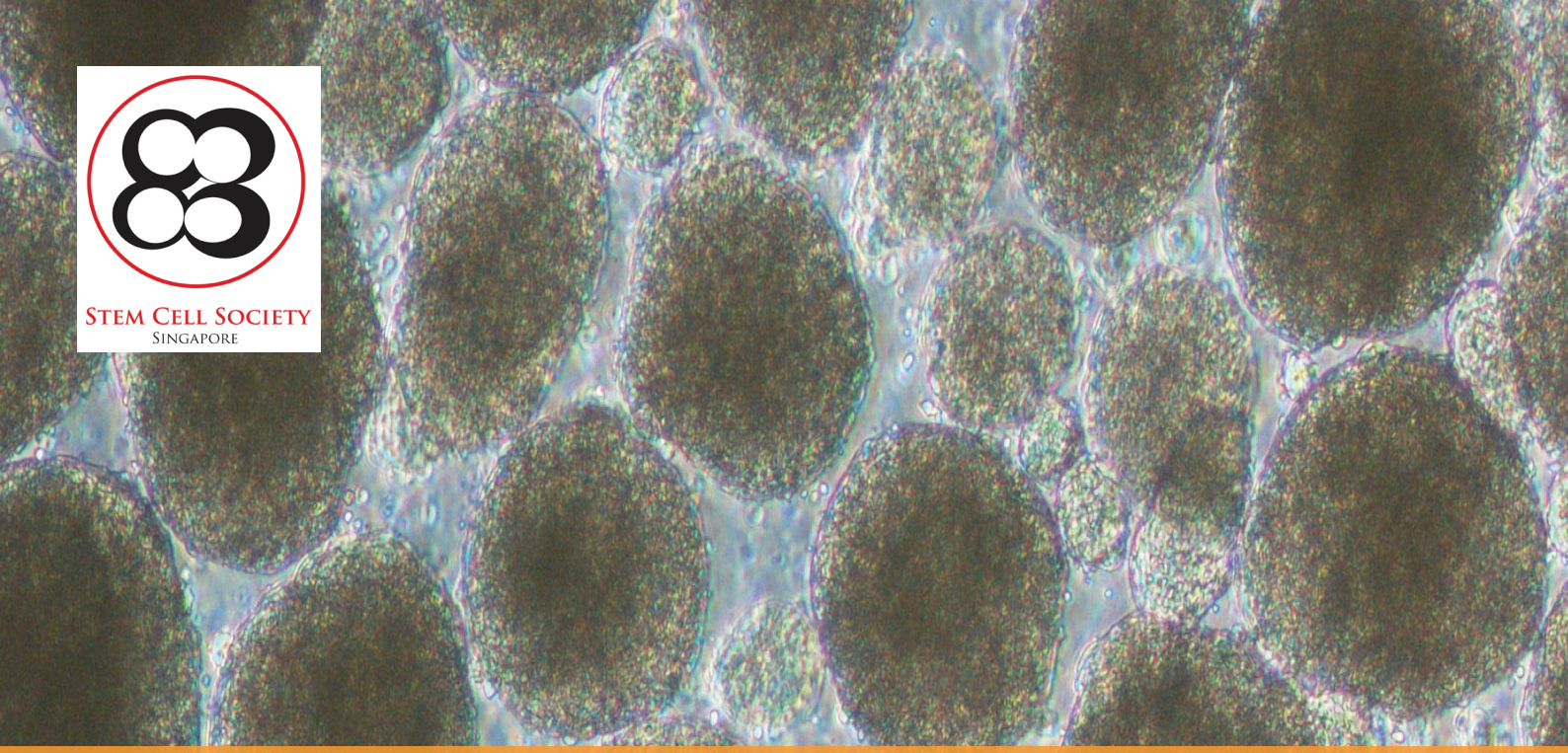


STEM CELL SOCIETY
SINGAPORE

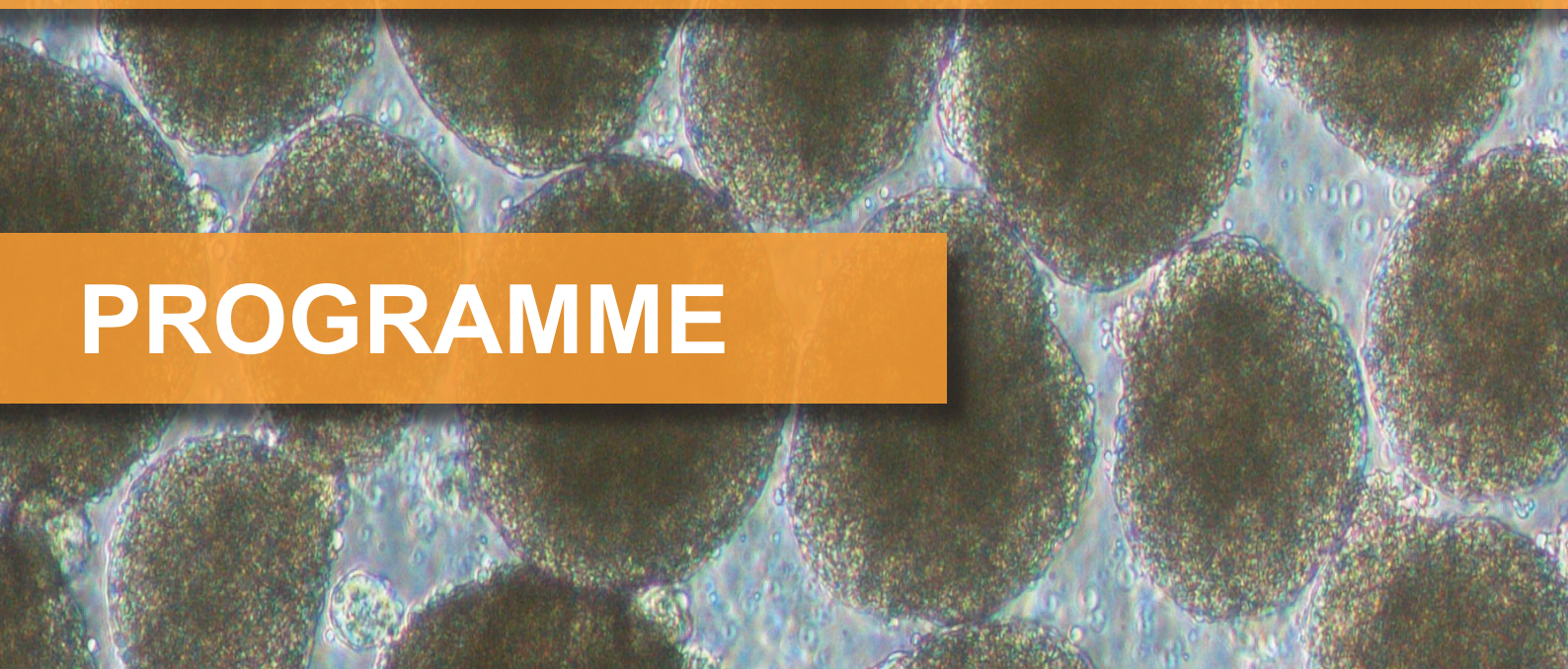
14th Annual Symposium 2022

Stem Cell Society Singapore

7 - 9 December

Clinical Sciences Building

LKCM, Novena Campus



PROGRAMME

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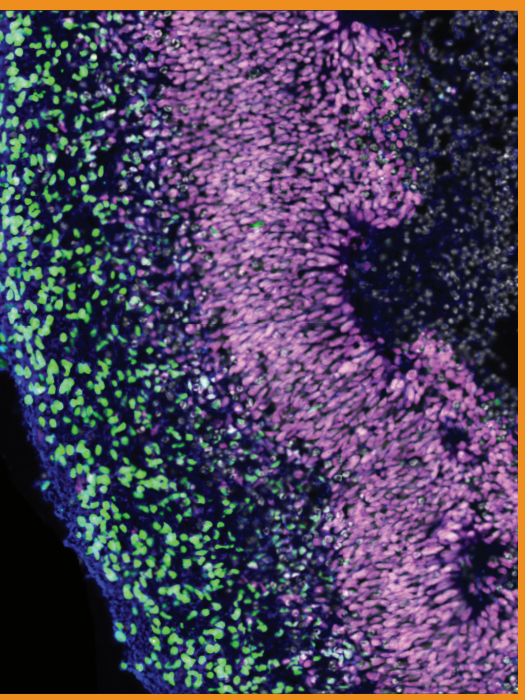
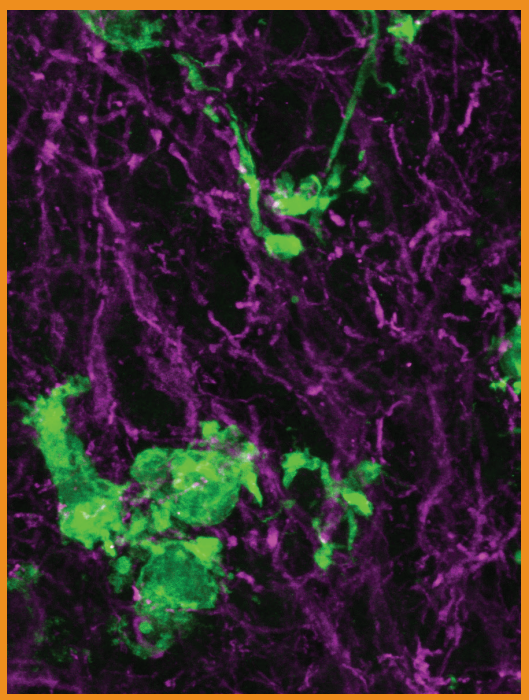
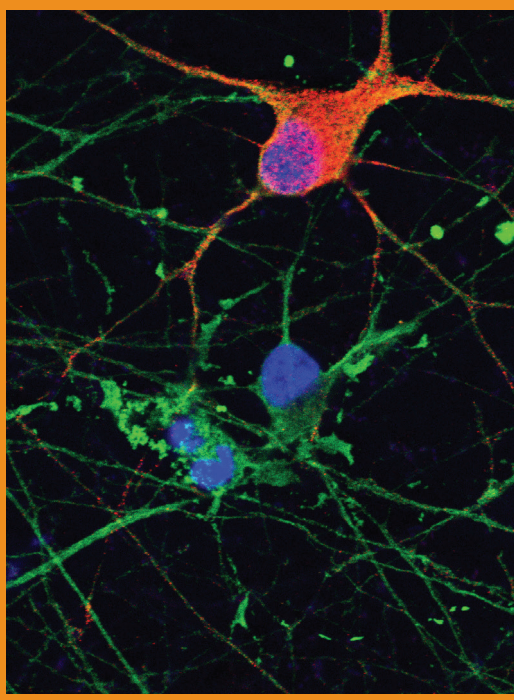

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Welcome Message

Dear Colleagues & Friends,

A very warm "Welcome" to the **14th Stem Cell Society Singapore Symposium 2022** in Singapore. After two years of having to hold our annual symposium virtually, we are indeed very pleased and relieved to be able to meet you in person and discuss with you face-to-face this year again. To be honest, we missed personal contact with you very much during the last two years.

The annual scientific symposium is the main event of the Stem Cell Society Singapore as it facilitates our goals of promoting stem cell research in Singapore by bringing together local and international professionals from different sectors.

Stem cell research has moved rapidly towards applications for the treatment of human diseases in the last couple of years and this year's programme showcases basic and translational research as well as industry and clinical efforts to bring stem cells to patients. The organizing committee has composed an excellent programme with outstanding overseas and local speakers. We are particularly excited to welcome three keynote speakers who are all international experts and opinion leaders in their fields: *Prof. Haifan Lin* (Yale School of Medicine, USA; President of ISSCR). *Prof. Lee Rubin* (Harvard Stem Cell Institute, USA), and Dr. *Shuibing Chen (*Cornell University, USA*)*. We have also invited a diverse set of experts who will contribute to sessions focusing on stem cells, ageing and rejuvenation, stem cell therapy, emerging technologies in cell manufacturing, stem cell models and precision medicine, and organoids as models for human diseases. Finally, this year we incorporated the Young Investigator Forum into the programme to celebrate the achievements of young researchers and discuss issues with their career progressions. We also have selected young researchers from abstracts to share their science stories and finally, there is a dedicated poster session to facilitate discussions among delegates. We are confident that the symposium will provide cutting-edge information on the status of the field and provides new insights into innovative approaches to advanced therapies.

A big "Thank you" goes out to our speakers who spent time and effort preparing and presenting their research to us. We would also like to acknowledge the vital support of our academic and industry sponsors that support us in holding our annual symposium. We would particularly like to thank Dr. Susan Lim for continuing to support the SCSS-Dr. Susan Lim Award for Outstanding Young Investigator. Last but not least, we are very grateful to our volunteers for helping to run the symposium smoothly.

Finally, we greatly look forward to your active participation and hope you enjoy interacting with your colleagues and friends in person again as well as enjoying the science shared with you.

The Organizing Committee

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Li Zeng (Co-chair)
Yie Hou Lee
Natasha Ng
Adrian Teo
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Yun Xia

14th STEM CELL SOCIETY SINGAPORE SYMPOSIUM

Clinical Sciences Building, LKCMedicine, Novena
7 - 9 December 2022

Young Investigators' Forum

on Day 1, 7th Dec 2022 (Wed)
Speaker talks from 1.45 pm to 5 pm
Networking from 5 pm to 6.30 pm

Featuring presentations from up and coming investigators

Keynote by

Shuibing Chen, Cornell University, USA
Human pluripotent stem cells, organoids and disease modelling

Jinyue Liu, Genome Institute of Singapore, A*STAR
Single-cell transcriptomics and RNA imaging to map autism pathophysiology

Lay Teng Ang, Stanford University, USA
A stem cell-based toolkit to study deadly biosafety level 4 viruses

Two talks on pluripotent stem cells from key industry players

Koji Tanabe, CEO, I Peace, USA
iPSC-driven inflection point in cell therapy

Kee Wah Lee, Bio-Rad Laboratories, Singapore
Using ddPCR to detect pluripotent stem cells with high sensitivity

Panel discussion on Stories about career transitions and the path to leadership in science

Shi Yan Ng
Principal Investigator
IMCB, A*STAR

Lisa Ooi
VP (Strategy)
Hummingbird Bioscience

Fong Ming Koh
Investment Principal
LYFE Capital

Ying Xim Tan
Director of Discovery Ops
Medisix Therapeutics

Networking & Cocktail Reception with a Speed dating segment

If you have not tried speed networking before, this is the time to try it out!

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For more information or to secure your spot, email Natasha at nhjng@imcb.a-star.edu.sg by 6th Dec 2022!

Acknowledgements

We are most thankful to our corporate members, supporters, and exhibitors for their generous support. Without their involvement, this symposium would not have been possible.

Please visit the exhibitors' booths, check out their technologies, lucky-draws, and interact with their representatives. Our supporters are a very important part of the stem cell and cell therapy ecosystem.

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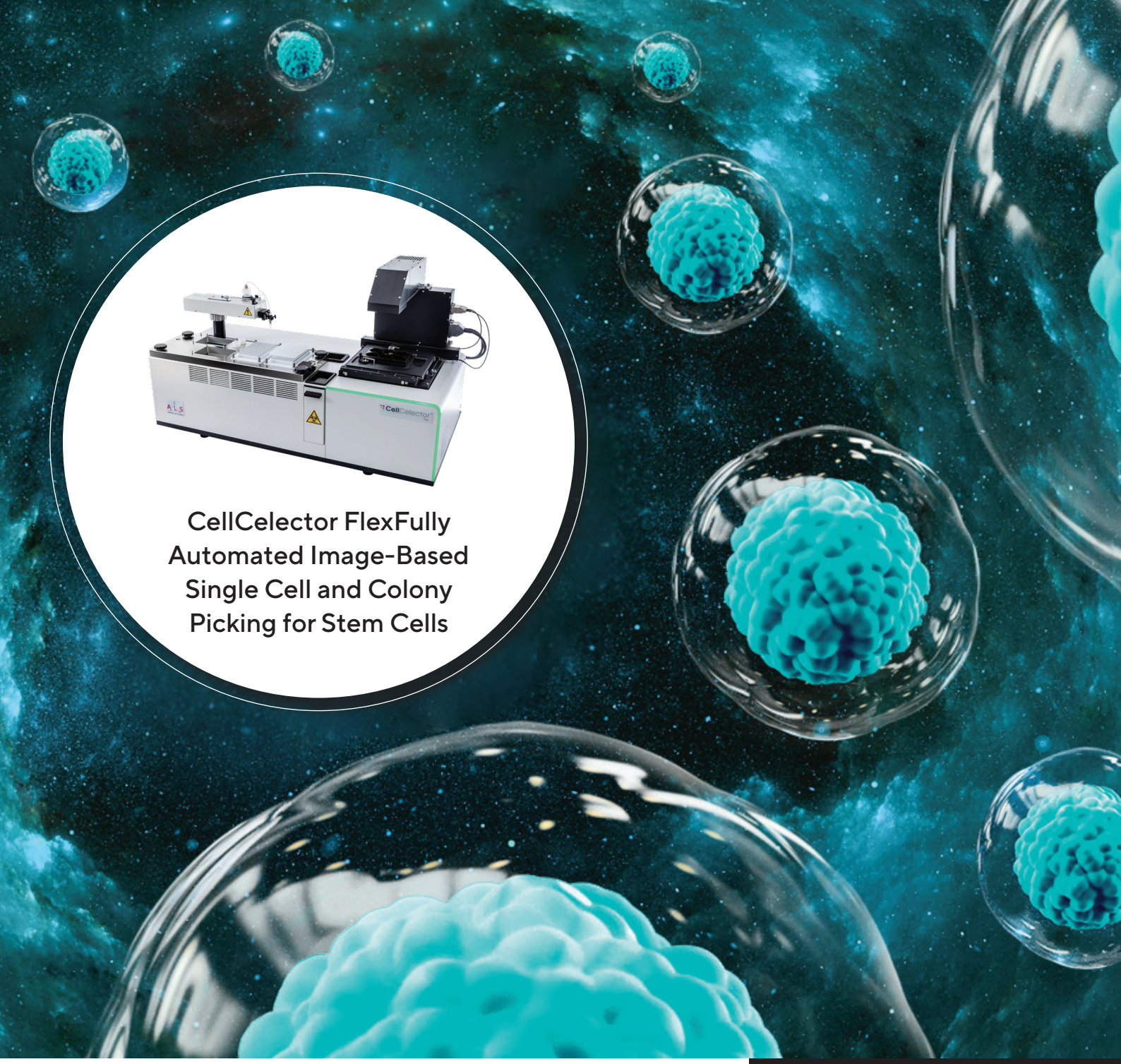

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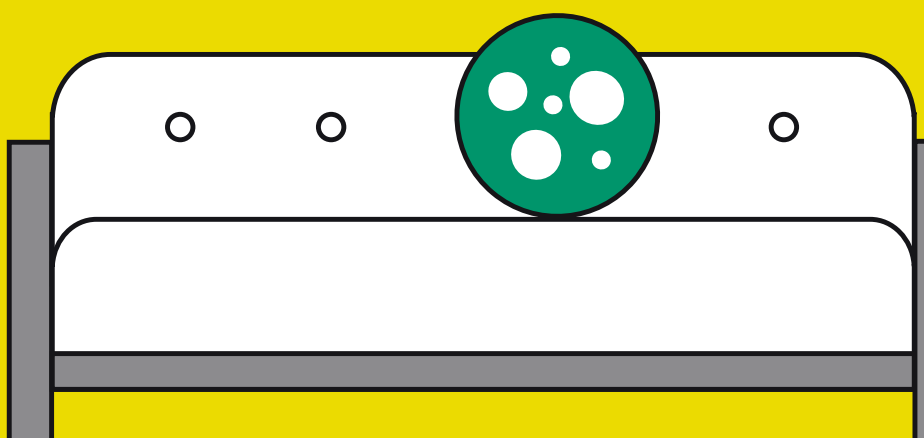

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Programme

9:00 – 9:10	**Welcome and opening** Ray DUNN, *President SCSS* & Jonathan LOH, *Chair of Organizing Committee*
9:10 -9:45	**Keynote lecture** *Chair*: Jonathan LOH, *Institute of Molecular and Cell Biology, Singapore* **The Piwi-piRNA pathway: a New World of gene regulation** Haifan LIN, *Yale School of Medicine, US*
9:45 - 11:10	**Session 1a. Precision Medicine and Stem Cell Models I** *Chair:* Christine CHEUNG**,** *LKCM, Singapore*
9:45 - 10:10	**Genome-scale CRISPR screening for LDLR-independent pathways regulating LDL uptake** Haojie YU, *National University of Singapore*
10:10 – 10:25	*Selected Abstract* **Characterization of LVV-transduced, in vitro-expanded anti-TIM3 CAR T cells** Wei-Xiang SIN, *Singapore-MIT Alliance for Research and Technology (SMART), Singapore*
10:25 – 10:45	**Industry Talk** **Leveraging genome editing of iPSCs to create disease models** Yu ZOU, *Thermo Fisher Scientific, Singapore*
10:45 – 11:10	**Implications of cancer cell state plasticity for therapeutic intervention** Wai Leong TAM, Genome Institute of Singapore
11:10- 11:40	BREAK
11:40 – 12:45	**Session 1b. Stem Cells, Ageing and Rejuvenation I** *Chair:* Li ZENG**,** *National Neuroscience Institute, Singapore*
11:40 – 12:05	**Combining stem cell rejuvenation and senescence targeting to synergistically extend lifespan** Nicholas Stanislaw TOLWINSKI, *Yale-NUS, Singapore*
12:05 – 12:20	*Selected Abstract* **Telomerase reverse transcriptase (TERT) loss-of-function results in motor neuron and astrocyte ageing** Jasmine HARLEY, *Institute of Molecular and Cell Biology, Singapore*
12:20 – 12:45	**Asian Immune Diversity Atlas (AIDA): Single cell analysis of human diversity** Kian Hong KOCK, *Genome Institute of Singapore*
12:45 – 13:45	LUNCH BREAK

13:45 – 16:55 **Session 2. Young Investigators' Forum**
Chair: Natasha NG**,** *Institute of Molecular and Cell Biology, Singapore*

13:45 – 14:10 **Keynote lecture**
Human pluripotent stem cells, organoids and disease modelling
Shuibing CHEN, *Cornell University, USA*

14:10 – 14:25 *Selected Abstract*
A stem cell-based toolkit to study deadly biosafety level 4 viruses
Lay Teng ANG, *Stanford Institute for Stem Cell Biology & Regenerative Medicine, USA*

14:25 – 14:40 Industry talk
Droplet Digital PCR provides high sensitivity in the detection of pluripotent stem cells
Kee Wah LEE, *Bio-Rad Laboratories (Singapore) Pte Ltd*

14:40 – 15:05 **Integrative analysis of single-cell transcriptomics with multiplexed RNA imaging for mapping autism pathophysiology**
Jinyue LIU, Genome Institute of Singapore

15:05– 15:35 BREAK

15:35 – 16:25 **Panel discussion – "Stories about career transitions and the path to leadership in science"**

Panellists:

Shi Yan NG, *Principal Investigator*, *Institute of Molecular and Cell Biology, Singapore*

Lisa OOI, Vice *President (Strategy)*, *Hummingbird Bioscience, Singapore*

Fong Ming KOH, *LYFE Capital, Singapore*

Ying Xim TAN, *Medisix Therapeutics, Singapore*

16:25 – 16:50 **iPSC-driven inflection point in the cell therapy industry**
Koji TANABE, *I Peace, USA*
Chair: Jonathan Loh, Institute of Molecular and Cell Biology, Singapore

16:50 – 18:30 YOUNG INVESTIGATORS' FORUM NETWORKING & COCKTAIL RECEPTION
With a SPEED DATING segment!

END OF DAY 1

9:00 – 10:25 **Session 3a. Stem Cells, Ageing and Rejuvenation II**
Chair: Li ZENG**,** *National Neuroscience Institute, Singapore*

9:00 – 9:25 **Interventional strategies to enhance longevity**
Brian KENNEDY, *National University of Singapore*

9:25 – 9:45 **Industry Talk**
Enhancing survival: Novel methods for stem cell colony selection and retrieval
Darius Cameron WILSON, *Sartorius Corporation*

9:45 – 10:00 *Selected Abstract*
Cord lining induced pluripotent stem cell-derived retinal pigment epithelium (CLiPS-RPE): A novel source for cell therapy for age-related macular degeneration (AMD)
Mayuri BHARGAVA, *Institute of Molecular and Cell Biology, Singapore*

10:00 – 10:25 **Communication between gut microbiota and organ function regulates Biological Ageing**
Sven PETTERSON, *National Neuroscience Institute, Singapore*

10:25 – 10:55 BREAK

10:55 – 12:00 **Session 3b. Precision Medicine and Stem Cell Models II**
Chair: Christine CHEUNG**,** *LKCM, Singapore*

10:55 – 11:20 **Integrating multi-omics data to improve understanding of T2D pathogenesis**
Xueling SIM, *National University of Singapore*

11:20 – 11:35 *Selected Abstract*
Modelling genetic polycystic kidney disease using human pluripotent stem cell-derived kidney organoids
Meng LIU, *Lee Kong Chian School of Medicine, Singapore*

11:35 – 12:00 **Singapore National Precision Medicine Program: Intersections with stem cell technologies**
Patrick TAN, *PRECISE, Singapore*

12:00 – 13:00 BREAK

Programme
Day 2, 8 Dec

13:00 – 13:35 **SCSS-Dr Susan Lim Award for Outstanding Young Investigator Presentation**
Chair: Ray DUNN, *Lee Kong Chian School of Medicine, Singapore*

Retinal Cell Therapy: the path to vision regeneration
Xinyi SU, *Institute of Molecular and Cell Biology, Singapore*

13:35 – 16:10 **Session 4. Stem Cell Therapy**
Chair: Jonathan LOH**,** *IMCB, Singapore*

13:35 – 13:50 *Selected Abstract*
Understanding the molecular signature of mesenchymal stem cell quality
Padmapriya SATHIYANATHAN, *Genome Institute of Singapore*

13:50 – 14:10 **Industry Talk**
Characterization of stem cells for cellular therapies
Anis LARBI, *Beckman Coulter Life Sciences, France*

14:10 – 14:35 **A learning journey of TCR-T therapy development**
Qi-Jing LI, *IMCB, Singapore*

14:35 – 15:05 BREAK

15:05 – 15:30 **Latest advances in ischemic and inflammatory disorders from Cytopeutics - focus on stroke and aGVHD**
Sze Piaw CHIN, *Cytopeutics Sdn. Bhd, Malaysia*

15:30 – 15:45 *Selected Abstract*
Secretome derived from hypoxia preconditioned mesenchymal stem cells promote cartilage regeneration and mitigate joint inflammation via extracellular vesicles
Yanmeng YANG, *National University of Singapore*

15:45 – 16:10 **From the skin to the joints: clinical translation, commercialisation and the future for cell-based regenerative therapies in orthopaedic surgery: where are we headed?**
Francis WONG, *Sengkang General Hospital, Singapore*

16:10 – 18:15 POSTER SESSION with Wine & Cheese

END OF DAY 2

9: 00 – 12:00 **Session 5. Emerging technologies in cell manufacturing**
Chair: Yie Hou LEE**,** *SMART, Singapore*

9: 00 – 9:25 **Label-free biophysical Critical Quality Attributes (CQAs) for cell therapy products**
Jongyoon HAN, *MIT/SMART, USA*

9:25 – 9:50 **Self-Sustaining Cell-Based Meat Paradigm: Making the muscle cell the serum source**
Alfredo FRANCO-OBREGON, National University of Singapore

9:50 – 10:15 **Label-free selection of mesenchymal stem cell subpopulations with more efficacious cartilage regenerative potential**
Zheng YANG, *National University Health System, Singapore*

10:15 – 10:45 BREAK

10:45 – 11:10 **Mechanical control of mammalian ovarian folliculogenesis**
Chii Jou (Joe) CHAN, Mechanobiology Institute, Singapore

11:10 – 11:35 **Development of mechanotechnologies for high content imaging of scaffolded organoids**
Virgile VIASNOFF, Mechanobiology Institute, Singapore

11:35 – 12:00 **Stem cell differentiation via self-induced physical confinement**
Andrew HOLLE, *National University of Singapore*

12:00 – 13:00 LUNCH BREAK

Programme
Day 3, 9 Dec

13:00 – 14:00 **Session 6. STEMCELL Technologies hosted session - Organoids as tools for disease modelling**
Chair: Riya SHARMA, *STEMCELL Technologies, Canada*

13:00 – 13:20 **Efficient generation of functionally relevant hPSC-derived hepatocytes and liver organoids for hepatotoxicity and liver biology modeling**
Riya SHARMA, STEMCELL *Technologies, Canada*

13:20 – 13:40 **Implementing personalised medicine for cystic fibrosis patients: Personalised stem cell derived organoids to select optimal treatment for each patient**
Shafagh WATERS, *University of South Wales, Australia*

13:40 – 14:00 **DIVERSITY: A national organoid biobank for translational research**
Nimmi BABY, *Singapore Translational Cancer Consortium (STCC)*

14:00 – 16:50 **Session 7. Organoid models for human diseases**

This session is supported by the
LEE KONG CHIAN SCHOOL OF MEDICINE (LKCM), SINGAPORE
Chair: Yun XIA**,** *Lee Kong Chian School of Medicine, Singapore*

14:00 – 14:25 **Modelling neural disorders using human pluripotent stem cells**
Shawn JE, *Duke-NUS Medical School*, Singapore

14:25 – 14:50 **Human pluripotent stem cell-derived pancreatic islet cells for human diabetes modelling and target discovery**
Natasha NG, *Institute of Molecular and Cell Biology, Singapore*

14:50 – 15:20 BREAK

15:20 – 15:45 **Modular hydrogels for organoid-based disease modelling**
Eileen GENTLEMAN, *King's College London, UK*

15:45 – 16:10 **Strategies to improve cardiovascular disease modelling in vitro**
Boon Seng SOH, *Institute of Molecular and Cell Biology, Singapore*

16:10 – 16:25 *Selected Abstract*
Retinal organoid: a model to study embryonic retinal development
Ying CHEN, *National University of Singapore*

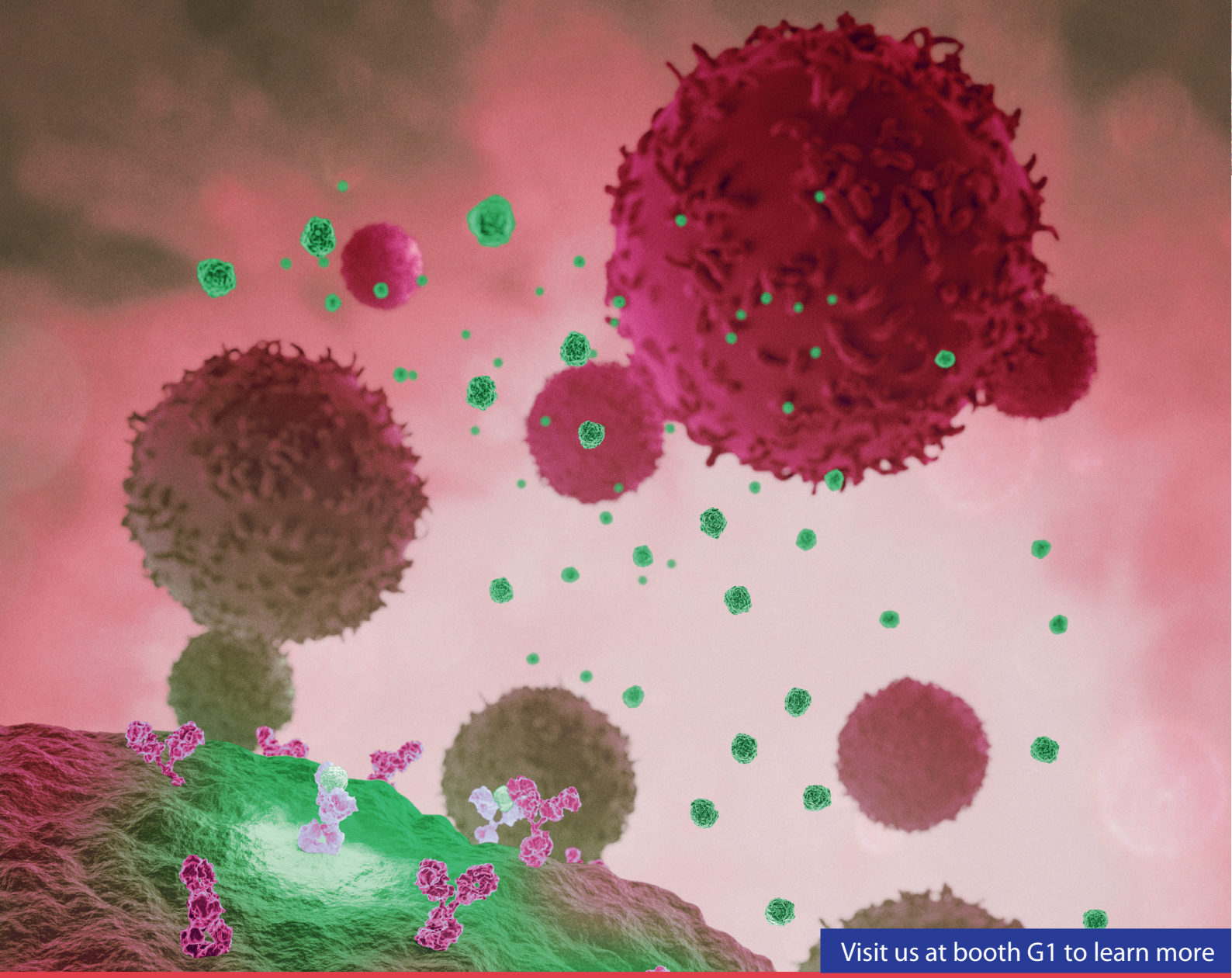
16:25 – 16:50 **Kidney organoids for disease models and therapeutic development**
Ryuji MORIZANE, *Harvard Medical School, USA*

16:50 – 17:25 **Keynote lecture**
Chair: Jonathan LOH, *Institute of Molecular and Cell Biology, Singapore*

De-aging the aging brain
Lee RUBIN, *Harvard Stem Cell Institute, USA*

17:25 – 17:40 AWARD PRESENTATION
CLOSING REMARKS
Chair: Li ZENG, *National Neuroscience Institute, Singapore*

END OF DAY 3 - FAREWELL



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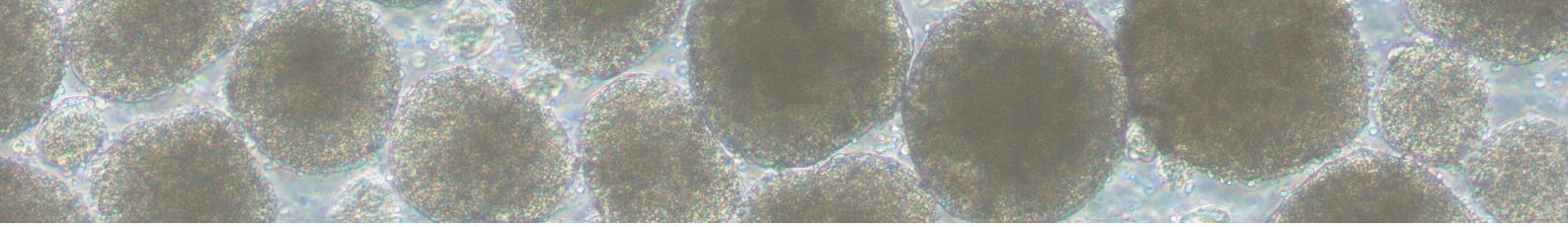
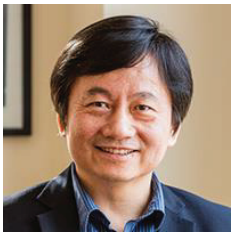


SPEAKER ABSTRACTS

Day 1

7 Dec 2022

KEYNOTE LECTURE



The Piwi-piRNA pathway: a New World of gene regulation

Haifan LIN

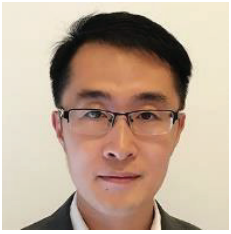
Yale Stem Cell Center, Yale University School of Medicine, USA

ABSTRACT

Small non-coding RNAs play key roles in gene regulation. In 1998, we discovered the argonaute-piwi gene family that is essential for stem cell self-renewal in both animal and plant kingdoms. Within this family, the argonaute (ago) subfamily is ubiquitously expressed. AGO proteins bind to siRNAs and miRNAs as central components of the RNAi and miRNA pathways. However, the piwi subfamily is mostly expressed in the germline and primitive stem cells. PIWI proteins binds to piRNAs (mostly 26-32 nucleotides). We and others discovered over 20 million different piRNAs in multiple organisms that correspond to all types of genomic sequences. They represent a vast new world of genetic regulation. I will present our recent work that revealed the crucial role of piRNAs in mediating the regulation of mRNA, lncRNA, and satellite RNA by transposons and pseudogenes, which represent a new paradigm of regulation that connect all major constituents of the genome.

BIO

Eugene Higgins Professor and founding Director, Yale Stem Cell Center. Dr. **Haifan Lin** studies the self-renewing mechanism of stem cells, stem cell-related cancers, and reproductive biology. He made key contributions to the demonstration of stem cell self-renewing division and the proof of the stem cell niche theory. He discovered the argonaut/piwi genes as the first gene family with essential stem-cell function highly conserved in both animal and plant kingdoms. He is also a discoverer of PIWI-interacting RNA (piRNA), a discovery hailed by Science as one of the 10 Breakthroughs in 2006. Recently, his lab demonstrated the crucial roles of the Piwi-piRNA pathway in epigenetic programming and post-transcriptional regulation of mRNA and lncRNA. Dr. Lin received more than 30 awards in his career. He is a Member of US National Academy of Sciences, a Member of the American Academy of Arts and Sciences, and a Foreign Member of the Chinese Academy of Sciences.



Genome-scale CRISPR screening for LDLR-independent pathways regulating LDL uptake

Haojie YU

National University of Singapore

ABSTRACT

Atherosclerotic cardiovascular disease (ASCVD) remains the leading cause of death worldwide, and lipid-lowering therapies that act independently of LDL Receptor (LDLR) are urgently needed. LDLR is the primary receptor in hepatocytes for clearing LDL particles from the blood. While fibroblasts isolated from HoFH patients showed almost completely abolished LDL uptake, the primary hepatocytes from these patients still bind and internalize LDL to a considerable extent, suggesting the existence of LDLR-independent pathways in hepatocytes to uptake LDL. In this study, we combined genome-scale CRISPR knockout screen and co-essentiality gene network analysis to discover the previously uncharacterized pathways in hepatocytes regulating LDLR-independent uptake of LDL. Our unbiased screen has discovered 802 genes whose disruption impacts hepatic LDL uptake. Through a co-essential gene network analysis pipeline, we further identified 37 gene modules significantly associated with LDL uptake. Functional validation of top hits has discovered many novel players regulating LDLR-independent uptake of LDL particles.

BIO

Haojie YU obtained a PhD in Molecular Biology from Nanyang Technological University, Singapore, after which he took the postdoc training at Harvard University and worked on metabolic diseases. Dr. Yu joined the National University of Singapore in 2020 and now he is an Assistant Professor in the Department of Biochemistry, Yong Loo Lin School of Medicine. His research aims to discover novel therapeutic strategies to tackle atherosclerotic coronary artery disease (CAD) and non-alcoholic steatohepatitis (NASH). He combines functional genomics and CRISPR/Cas9-based functional screening to understand how alterations in lipid metabolism and trafficking contribute to NASH and atherosclerotic plaque progression and regression.

Abstract selected talk

Characterization of LVV-transduced, in vitro-expanded anti-TIM3 CAR T cells

Wei-Xiang SIN[1], Faye CHEUNG[1], Sandy LEE[2], Dedy SANDIKIN[1], Denise TEO[1], Faris KAIRI[1], Cheng-I WANG[2], Michael BIRNBAUM[1,3]

*[1]Critical Analytics for Manufacturing Personalized-Medicine (CAMP), Singapore-MIT Alliance for Research and Technology (SMART), Singapore; [2]Singapore Immunology Network (SIgN), Agency for Science, Technology and Research (A*STAR), Singapore; [3]Department of Biological Engineering, Massachusetts Institute of Technology (MIT), USA*

ABSTRACT

T-cell immunoglobulin and mucin domain 3 (TIM3) is highly expressed on acute myeloid leukemia (AML) blasts and leukemic stem cells (LSCs), and not expressed on normal hematopoietic stem cells, granulocytes, naïve lymphocytes, and most normal non-hematopoietic tissues. The STAMP/IMPACT program has designed a second-generation anti-TIM3 CAR to target minimal residual disease (MRD) in AML, and showed that transient expression of anti-TIM3 CAR by mRNA electroporation can endow T-cells with anti-leukemic activity against AML blasts and LSCs. However, TIM3 is also expressed on activated lymphocytes, leading to concerns over T cell fratricide. We aimed to characterize the biological properties of ex vivo-cultured T-cells displaying stable expression of anti-TIM3 CAR via lentiviral vector (LVV) transduction. We found that, compared with prototypical anti-CD19 CAR T-cells, anti-TIM3 CAR T-cells exhibited lower viability, lower proportions of naïve T-cells, higher proportions of terminally differentiated T-cells, higher basal expression of Perforin and Granzyme B in the absence of exogenous stimulation, and significant non-specific cytolytic activity against a cell line that do not express TIM3. Taken together, these suggest that interactions between TIM3 and the anti-TIM3 CAR exist among T-cells in culture, resulting in continuous stimulation as well as T cell fratricide.

Leveraging genome editing of iPSCs to create disease models

Yu ZOU

Thermo Fisher Scientific

ABSTRACT

Human pluripotent stem cells (PSC), such as human embryonic stem cell (hESC) or induced pluripotent stem cell (hiPSC) are capable of indefinite self-renewal and differentiated into essentially all cell types. Equipped with the modern genome editing technology e.g., CRISPR and TALENs, iPSCs are widely used in disease modelling and drug discovery. However, due the stringent requirement for PSC maintenance, delivery of editing tools into the cells and following selection of clonal populations are usually challenging. In this presentation, we will introduce Thermo Fisher's PSC workflow and genome editing workflow and highlight some of Thermo Fisher's tools with proven use in PSC editing and cell line generation. We will also share a case study of our customer using our technology in iPSC disease modeling.

BIO

Yu Zou received his PhD in 2014 in National University of Singapore. His work involves analysis of telomere length and telomerase activity of different human embryonic stem cell lines and their differentiated progeny cell types. After receiving his PhD, he worked in Institute of Medical Biology, Agency of Science Technology and Research (IMB, A*STAR) on the efficacy of human Wharton's jelly stem cells for wound healing on a 3D in vitro skin model. Zou Yu joined Thermofisher in March 2020 as Technical Sales Specialist, supporting the sales of a wide range of products in Cell Biology and Synthetic Biology portfolio.

Implications of cancer cell state plasticity for therapeutic intervention

Wai Leong TAM

Genome Institute of Singapore, Singapore

ABSTRACT

Cancer progression is orchestrated by complex alterations in cellular behaviors that enable the survival of, and colonization by, cancer cells in the human body. Disturbances in cell states underlie disease progression and clinical outcomes. Underpinning the control of cancer cell states are metabolic reprogramming events, i.e., why are the nutritional needs of cancer cells unique or different? Many fundamental cancer pathways are now known to impinge on cell metabolism, and strategies focusing on disrupting specific metabolic processes to control disease progression have gained considerable interests. Leveraging on functional genomic approaches, and in close collaboration with clinician partners, our research program group seeks to gain insights into the precise control of cancer cell states, and specific targeting of metabolic pathways, for the development of selective therapeutics against cancer resistance and metastasis.

BIO

Wai Leong Tam received his PhD at the Genome Institute of Singapore, where he worked on uncovering the bases for the pluripotency in embryonic stem cells and iPSCs. He undertook his postdoctoral training at the Whitehead Institute in MIT, where he concentrated on cancer stem cells and metastasis. Subsequently, he joined GIS and CSI as a Principal Investigator and his lab focuses on uncovering and interrogating the emerging paradigms of cancer metabolism and cell state transition.

Combining stem cell rejuvenation and senescence targeting to synergistically extend lifespan

Nicholas Stanislaw TOLWINSKI

Yale-NUS, Singapore

ABSTRACT

Why biological age is a major risk factor for many of the most important human diseases remains poorly understood. We know that, as organisms age, stem cell pools are exhausted while senescent cells progressively accumulate. I will discuss how induction of pluripotency via expression of Yamanaka factors and clearance of senescent cells can ameliorate aspects of cellular and physiological aging, and how combination therapies work to extend lifespan and healthspan.

BIO

Nicholas TOLWINSKI is an Associate Professor at Yale-NUS. He received a PhD from Princeton University in Molecular Biology in 2004. After completing his PhD, he became the first Frank A. Howard Scholar in the Developmental Biology at Memorial Sloan-Kettering Cancer Center. His laboratory specializes in analyzing the early embryonic development of Drosophila. His research focuses on modeling systems of signal transduction organizing groups of cells into tissues, and what goes wrong with these processes leading to cancers. The laboratory's primary focus is on Wnt signaling in development and aging.

Abstract selected talk

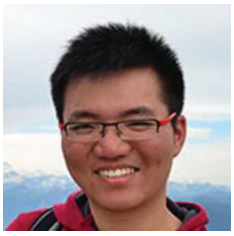
Telomerase reverse transcriptase (TERT) loss-of-function results in motor neuron and astrocyte ageing

Jasmine HARLEY[1], Munirah Mohamad SANTOSA[1,2], Chong Yi NG[1], Valerie Jingwen LIM[1], Dinesh KUMAR[2], Derrick Sek Tong ONG[2], Shi-Yan NG[1]

*[1]Institute of Molecular and Cell Biology, A*STAR Research Entities, Singapore; [2]Department of Physiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore*

ABSTRACT

Telomerase reverse transcriptase (TERT) functions to maintain telomere length, which are specialised protective caps at the end of chromosomes. Telomere attrition is a hallmark of ageing and critically short telomeres induce senescence and prevent proliferation. As age is the biggest risk factor for neurodegenerative diseases, understanding the role of TERT in the neural cells may have important implications in understanding disease mechanisms. To investigate the role of TERT in neural cells of the spinal cord, TERT loss-of-function iPSCs were generated by CRISPR/CAS9 and differentiated into spinal cord motor neurons and astrocytes. TERT mutant motor neurons displayed hallmarks of ageing, including increased DNA damage, cellular senescence and decreased mitochondrial function. TERT mutant astrocytes also exhibited age-associated characteristics, including an increase in cell size, an increase in GFAP expression and increased DNA damage. This shows TERT loss-of-function can be used to induce an in vitro model of ageing and provides an important tool to investigate age-related decline and neurodegenerative diseases.



Asian Immune Diversity Atlas (AIDA): Single cell analysis of human diversity

Kian Hong KOCK[1], Shyam PRABHAKAR[1, 2]

[1]Spatial and Single Cell Systems Domain, Genome Institute of Singapore, Agency for Science, Technology and Research, Singapore; [2]Associate Investigator, Cancer Science Institute of Singapore

ABSTRACT

The Asian Immune Diversity Atlas (AIDA) consortium is mapping immune cell variation within and across Asian populations. We have completed data generation for AIDA Phase 1 by genotyping and performing single cell RNA-seq on >500 healthy individuals from 5 ancestry groups across Singapore, Japan and South Korea. The Singapore cohort, which is a subset of the HELIOS cohort within Singapore's National Precision Medicine Programme, includes individuals of Chinese, Malay and Indian ancestry. All three cohorts are gender-balanced and span a range of ages. The AIDA dataset affords a unique glimpse into the effects of age, sex and ancestry on cellular proportions and cell-type-specific gene expression. The data also reveal the effects of individual genetic variants on gene expression, within and across demographic groups. In addition to advancing our understanding of human diversity, AIDA will serve as a healthy control dataset for future single-cell studies of immune disease mechanisms and markers.

BIO

Kian Hong Kock is a Research Fellow at the Genome Institute of Singapore, A*STAR, and leads the GIS Asian Immune Diversity Atlas (AIDA) team. He obtained a BA in Natural Sciences from the University of Cambridge, and a PhD in Biological and Biomedical Sciences from Harvard University. Kian Hong is an A*STAR National Science Scholar, and his research interests include understanding the landscape of transcriptional regulatory variation and cellular variation underlying diverse human populations using both experimental and computational approaches.

KEYNOTE LECTURE

Human pluripotent stem cells, organoids and disease modelling

Shuibing CHEN

Department of Surgery, Weill Cornell Medicine

ABSTRACT

The major research interest in the Chen Laboratory is to manipulate stem cell fate using chemical and biological approaches and to generate functional tissues and organs that can be used for translational research. Our current main focus is on human pluripotent stem cells (PSCs), including human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs). We combine our knowledge of stem cell biology, developmental biology, chemical biology, medicine chemistry and tissue engineering to derive functional cells, tissues and organs from human pluripotent stem cells. Our long-term goal is to apply patient specific PSC-derived tissues or organs for replacement therapy and build up “disease in a dish” platforms for drug discovery.

BIO

Shuibing CHEN is the Kilts Family Professor and the Director of the Diabetes Program in Department of Surgery at Weill Cornell Medicine. She received my B.S. and M.S. from Tsinghua University Then, she pursued my PhD under the advisement of Dr. Peter G. Schultz at the Scripps Research Institute. After graduation, she joined Dr. Doug Melton’s laboratory at Harvard University. The major research interest in the Chen Laboratory focuses on human pluripotent stem cell-derived organoids for disease modeling and drug screening. In response to the COVID-19, Dr. Chen created a panel of hPSC-derived cells/organoids to study SARS-CoV-2 infection. Part of this work has been published on Nature, Cell Stem Cells, Cell Metabolism, Circulation Research, etc. She has received many awards including New York Stem Cell Foundation Robertson Investigator, American Diabetes Association Innovative Award, NIH Director’s New Innovator Award, and ISSCR Dr. Susan Lim Award for Outstanding Young Investigator, etc.

Abstract selected talk

A stem cell-based toolkit to study deadly biosafety level 4 viruses

Lay Teng ANG

Stanford Institute for Stem Cell Biology & Regenerative Medicine, Stanford University School of Medicine, USA

ABSTRACT

Stem cell research endeavors to generate specific subtypes of classically defined 'cell types.' Here, we generate >90% pure human artery or vein endothelial cells from pluripotent stem cells within 3–4 days. We specified artery cells by suppressing vein-specifying signals and vice versa. These cells modeled viral infection of human vasculature by the Nipah virus, which emerged in Malaysia and Singapore in 1998-1999, are extraordinarily deadly (57%–59% fatality rate) and require biosafety-level-4 containment. Generating pure populations of artery and vein cells highlighted that the Nipah virus preferentially infected arteries; arteries expressed higher levels of their viral-entry receptor. Virally infected artery cells fused into giant syncytia, which rapidly died. Despite infecting arteries and occupying 6%–17% of their transcriptome, the Nipah virus largely eluded innate immune detection, minimally eliciting interferon signaling. We thus efficiently generate artery and vein cells, introduce stem-cell-based toolkits for biosafety-level-4 virology, and explore the arterial tropism and cellular effects of the Nipah virus.

Droplet Digital PCR provides high sensitivity in the detection of pluripotent stem cells

Kee Wah LEE

Bio-Rad Laboratories (Singapore) Pte Ltd

ABSTRACT

Induced pluripotent stem cells (iPSCs) are a valuable resource of regenerative medicine because of their capacity for pluripotent differentiation and unlimited self-renewal. There are an increasing number of stem cell therapies and clinical trials. However, the quantities of iPSCs must be carefully measured and controlled to prevent formation of malignant tumors after implantation. Although flow cytometry could be used to detect specific antigens of PSC, the high background of the negative control could limit its sensitivity. Using the Bio-Rad Droplet Digital PCR (ddPCR) System, iPSCs specific gene markers could be detected with a sensitivity of as low as 0.001%. Besides, ddPCR is also useful for the precise and quantitative measurements of genome editing events in iPSCs to enable patient-specific studies of pathological mutations. Join us at our talk to find out how ddPCR enables sensitive detection of iPSCs.

BIO

Kee Wah Lee is the regional Scientific Affairs Specialist for Digital Biology Group at Bio-Rad Laboratories. Kee Wah graduated with a Ph.D from the National University of Singapore (Singapore), specializing in crosstalk between different sub-population of cancer cells. He has also obtained his M.Sc and B.Sc Honors' degrees from the University of Nottingham (UK) and University Malaya (Malaysia), respectively. Kee Wah started his career as a postdoctoral fellow at the Department of Anatomy, National University of Singapore, working mainly on cancer biomarker discovery and validation.

Integrative analysis of single-cell transcriptomics with multiplexed RNA imaging for mapping autism pathophysiology

Jinyue LIU

Genome Institute of Singapore

ABSTRACT

Autism spectrum disorder (ASD) is the top disease burden among children in the developed world. Patients struggle with social communication, and display repetitive behaviors and restricted interests. While some can live independently, many face severe disabilities and require life-long support. Current drugs only alleviate co-morbidities and do not target core symptoms. To identify therapeutic opportunities, we sought novel cellular and molecular factors that may contribute to ASD. We integrated multiplexed RNA imaging with single-cell RNA sequencing to reconstruct the spatial molecular architecture of human brain organoids derived from typical and ASD subjects. We generated spatial maps for up to 557 genes over 838,042 cells across 47 tissue sections and observed profound structural derangement within ASD organoids. Specific cellular identities were mis-localized and altered in transcriptomic programmes underlying differentiation and synapse organization. This study exemplifies the use of spatial transcriptomics with stem-cell based modelling for unravelling pathophysiology of complex human disorders.

BIO

Jinyue Liu, Ph.D. heads the Laboratory of Single-Cell Spatial Neuromics at Genome Institute of Singapore. Her lab applies and develops nucleic-acid based technologies to decode the human brain during health and disease. She obtained her Ph.D. in Neurobiology at Harvard University under the mentorship of Prof Joshua Sanes and completed her undergraduate studies at University of Cambridge under the A*STAR National Science Scholarship. In recognition of her pioneering work and significant contributions in inspiring and shaping the local technology landscape, she was listed as one of the Singapore 100 Women in Tech in 2021.

PANEL DISCUSSION - PANELLISTS

Shi Yan NG

Institute of Molecular Biology, Singapore

BIO

Shi Yan Ng is a Principal Investigator at the Institute of Molecular Biology, A*STAR where she also holds a concurrent appointment as Director of Graduate Affairs (BMRC). Research in her lab centres around metabolic dysfunctions in human neurological diseases and using patient iPSCs to evaluate potential therapeutics. Shi Yan is also a recipient of a number of scientific awards, such as the National Research Foundation Fellowship and the L'Oréal For Women in Science National Fellowship. Prior to her current position, Shi Yan was a junior PI in IMCB after completing her postdoctoral stint in Professor Lee Rubin's lab at Harvard University.

Lisa OOI

Hummingbird Bioscience, Singapore

BIO

Lisa Ooi is the Vice President of Strategy at Hummingbird Bioscience. Lisa has more than 18 years of experience in the biomedical industry in the areas of stem cell biology, oncology/hematology, preclinical and clinical development, as well as large-scale private and public research partnerships and investment promotion. She has previous experience at the Singapore Economic Development Board, ASLAN Pharmaceuticals, Bayer Healthcare, the Agency for Science Technology and Research (A*STAR), and served on the boards of multiple biomedical entities such as Precision Health Research, Singapore (PRECISE) and the Singapore Clinical Research Institute (SCRI). Lisa holds a BSc in Biochemistry from the University of Wisconsin-Madison, a MSc and PhD from Stanford University in Chemical Engineering and Chemical and Systems Biology.

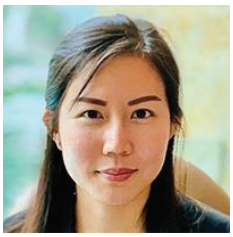
PANEL DISCUSSION - PANELLISTS

Foong Ming KOH

LYFE Capital, Singapore

BIO

Fong Ming Koh is a Principal at LYFE Capital, a global healthcare investment firm targeting early- to growth-stage opportunities in biopharmaceuticals, medical devices and diagnostics. Prior to joining LYFE, Fong Ming was the Head of Life Sciences at Heritas Capital, a Singapore-based healthcare-focused impact fund manager investing in biotech, digital health, food tech and more. A developmental biologist by training, he has extensive research experience spanning pluripotent stem cells, epigenetics and cancer. Fong Ming received his Ph.D. in Biomedical Sciences from the University of California, San Francisco, and a B.Sc. in Molecular Biology from the University of California, San Diego, with support from the A*STAR National Science Scholarship.



Ying Xim TAN

MediSix Therapeutics, Singapore

BIO

Ying Xim Tan is the Director of Discovery Operations at MediSix Therapeutics, a preclinical immune cell engineering company dedicated to developing novel T cell therapies for T cell malignancies and autoimmune diseases. She joined MediSix in 2018 as a Research Scientist and currently leads the Discovery team's research efforts to advance MediSix's pipeline. Ying Xim has over 15 years of experience in immunology and cancer biology research. She earned her BSc in Molecular and Cell Biology from the University of California, Berkeley and her PhD in Immunology from the University of California, San Francisco, where she studied T cell receptor signalling. Prior to working at MediSix, she was a Research Fellow at the Institute of Molecular and Cell Biology, studying the role of methyltransferases in liver biology and cancer.

iPSC-driven inflection point in the cell therapy industry

Koji TANABE, Kenta SUTO, Sakura TAMAKI, Priyanka SONI

I PEACE, USA

ABSTRACT

I Peace combines cutting-edge science with deep manufacturing expertise, providing GMP iPSC and iPSC-derived cell product manufacturing for clinical use globally. Our licensed (PMDA: Japan) and registered (FDA: USA) manufacturing facility is compliant with 21 CFR 210/211, EudraLex, Japanese regulations, and other relevant guidelines. We have developed a proprietary method to produce iPSCs from multiple donors in parallel, minimizing cross-contamination concerns for manufacturing iPSCs at scale for clinical use. Our highly efficient and rapid reprogramming methodology can achieve nearly ideal reprogramming efficiency with most cells giving rise to pluripotent marker-positive cells within 10 days, confirmed with single cell RNA sequencing and ATAC sequencing analyses. We are expanding our service and product offering including establishment of iPSC-derived cells and direct reprogramming, having successfully demonstrated direct reprogramming of dermal fibroblasts into oligodendrocyte precursor cells by overexpression of four genes.

BIO

Koji Tanabe engaged in iPSC research at Kyoto University under Nobel Laureate Dr. Shinya Yamanaka. He engaged in the research on induced pluripotent stem cells (iPSCs) and was the second author of the paper that reported the first successful establishment of human iPSCs. After earning a doctoral degree in 2013, he moved to Stanford University, working under Dr. Marius Wernig to broaden the scope of his research, particularly in the areas of reprogramming of cells. In 2015, Koji founded I Peace, Inc. in Palo Alto, CA. to bring everyone “Peace of Mind with iPSCs.” In 2020, the company opened its cell manufacturing facility in Kyoto, Japan, to provide cGMP iPSCs and iPSC-derived cells to cell therapy developers. Koji is the recipient of the “World Biz Magazine Top 100 Innovation CEOs 2021,” “Japan Entrepreneur Award 2022”, and “BioSpectrum Asia Excellence Awards 2022.”

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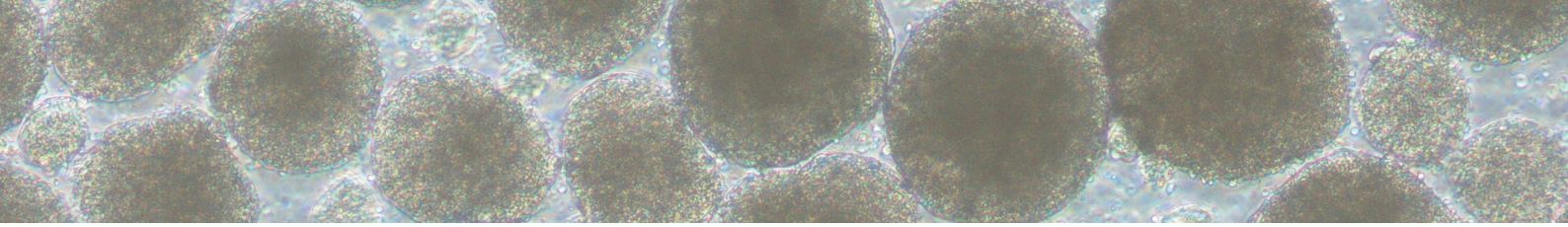

SPEAKER ABSTRACTS

Day 2

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SCAN ME

Interventional strategies to enhance longevity

Brian KENNEDY

National University of Singapore

ABSTRACT

Ageing research has slowly gained momentum over the last three decades and now become a mature field of scientific endeavor. This has occurred only just in time, as aging is likely the largest medical challenge of the 21st Century. Ageing is now recognized as the biggest risk factor for the onset of chronic diseases and the biggest predictor of complications in many infectious diseases. Given that over 20% of the population will be over 65 years of age in the not-too-distant future, it is imperative that strategies are developed to slow or reverse ageing processes. Fortunately, as a result mostly of research in animal models, there are no shortages of interventions that have the potential to extend human healthspan. Delaying ageing in animals is one thing, but validating them for efficacy in humans is entirely different. Here, I will discuss strategies to “get human” illustrating possible interventions that have a likelihood of success and discussing the possible clinical endpoints to test them in the clinic. People have tried to delay or reverse ageing for millennia, and it certainly appears possible that this nearly ageless quest can be achieved in the near future.

BIO

Brian Kennedy is internationally recognized for his research in the basic biology of aging and as a visionary committed to translating research discoveries into new ways of detecting, delaying, preventing and treating human aging and associated diseases. He is a Distinguished Professor in Biochemistry and Physiology at the Yong Loo Lin School of Medicine at National University Singapore and serves as Director of (1) the Centre for Healthy Longevity at the National University Health System, (2) the Healthy Longevity Translational Research Programme and (3) the Asian Centre for Reproductive Longevity and Equality at NUS Medicine. Collectively, NUS aging research seeks to demonstrate that longevity interventions can be successfully employed in humans to extend healthspan, the disease-free and highly functional period of life. From 2010 to 2016, Dr. Kennedy was the President and CEO of the Buck Institute for Research on Aging and he maintained a professorship there through 2020. Dr. Kennedy has an adjunct appointment at the Department of Biochemistry at the University of Washington, where he was a faculty member from 2001 to 2010. In addition, Dr. Kennedy is also actively involved with a number of Biotechnology companies. In addition, Dr. Kennedy serves as a Co-Editor-In-Chief at Aging Cell. Finally, Dr. Kennedy has a track record of interaction in China, where he was a Visiting Professor at the Aging Research Institute at Guangdong Medical College from 2009 to 2014. His Ph.D. was performed in the laboratory of Leonard Guarente at M.I.T., where he published the first paper linking Sirtuins to aging.

Enhancing survival: Novel methods for stem cell colony selection and retrieval

Darius Cameron WILSON

Sartorius Corporation

ABSTRACT

Human induced pluripotent stem cells (hiPSCs) are an invaluable resource in the field of regenerative medicine, with hiPSC reprogramming providing a powerful tool to aid drug discovery and cell therapy-based strategies. However, both hiPSCs and human embryonic stem cells (hESCs) can be difficult to culture, with significant questions raised over cellular survival following manual dissociation and enzymatic passaging, long term pluripotency retention, and genetic drift. A variety of tools are therefore required to regularly characterise and assay cells without influencing biological activity. This presentation will therefore highlight a number of novel imaging tools which can be utilized across different pluripotent workflows to help maintain high levels of cellular viability and pluripotency in the fields of colony growth profiling, colony isolation and single cell selection.

BIO

Specializing in the early identification of disease progression resulting from a dysregulated host response to localized bacterial or viral infections, **Darius Cameron. Wilson** is the author of 14 patents and numerous publications in fields such as sepsis, gastrointestinal surgery, trauma related complications, antibiotic guidance, and intensive care medicine. Dr. Wilson initially gained his PhD in physiology and pharmacology and an MBA from Glasgow Caledonian University, and is currently head of product management at Sartorius in the field of single cell selection and retrieval.

Abstract selected talk

Cord lining induced pluripotent stem cell-derived retinal pigment epithelium (CLiPS-RPE): A novel source for cell therapy for age-related macular degeneration (AMD)

Mayuri BHARGAVA[1,4], Bhav Harshad PARIKH[1], Paul BLAKELEY[1,2], Regha KAKKAD[1,2], Zengping LIU[1,2,3], Xinyi SU[1,2,3,4]

*[1]Institute of Molecular and Cell Biology (IMCB), Agency for Science, Technology and Research (A*STAR), Singapore; [2]Department of Ophthalmology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore; [3]Singapore Eye Research Institute, Singapore; [4]Department of Ophthalmology, National University Hospital, Singapore*

ABSTRACT

Background: Replacing dysfunctional RPE with healthy tissue is a promising treatment for AMD. Since oxidative stress leads to RPE mitochondrial dysfunction causing bioenergetic crisis, it is imperative that cell source can withstand stress. Current RPE cell products in pre-clinical trials from human embryonic stem cells (hESCs) and skin-derived human induced pluripotent stem cells (skin-hiPSCs) are associated with immunogenicity, mutagenicity, ethical concerns and lack data on bioenergetics. Novel source of cord lining stem cells can be obtained non-invasively, without ethical concerns, are somatically pristine, with low immunogenicity. **Objectives**: Assessment of CLiPS-RPE characterization, bioenergetics, and immune response after transplantation into wild-type rabbit eyes, without immunosuppression. **Methods**: Characterization of stem cell RPE (SC-RPE: CLiPS, skin-hiPSCs and hESCs) was studied using gene expression and protein localization. Bioenergetics in basal and stressed conditions was measured by seahorse assay. All RPE lines were xenografted in rabbit eyes. **Results**: CLiPs-RPE displayed pigmented cobblestone-like morphology, expressed key RPE genes and protein, demonstrated functional barrier activity, active phagocytosis, and polarized functions. Bioenergetically, CLiPS-RPE had higher oxidative potential. This was corroborated by oxidative stress test, where CLiPS-RPE demonstrated higher resistance, outperforming other RPE lines. Upon xenotransplantation, only rabbits with CLiPS-RPE displayed better host-graft response with absence of severe immune rejection.

Communication between gut microbiota and organ function regulates Biological Ageing

Sven PETTERSON

Principal Investigator, National Neuroscience Institute, Singapore; Senior Professor, National University of Singapore; Senior Fellow, Canadian Institute For Advanced Research, CIFAR

ABSTRACT

Singapore are experiencing a rapid increase of old human beings. While ageing is always associated with reduced organ function and increased risk to contract disease, the speed of organ decline is highly variable between individuals and subject to regulation by non-genetic factors, including the indigenous gut microbiota. Gut microbes have guided the evolution and organ development of all species through natural selection and fitness. Therefore, gut microbiota influences mammalian physiology, across the life span of the host in a diet and sex dependent manner. Through developmental programming acting in concert with maternal microbes and their metabolites, a new human being is formed. The new human body, with its highly specialized organs, establishes interorgan crosstalk and metabolic homeostasis tuned by gut microbes and diet in an age-dependent manner. That is, any age-related change in organ function or interorgan communication, by age or diet, will be sensed by gut microbiota who reciprocates by alteration in composition, richness, and secretion of microbe secreted molecules, including for example tryptophan metabolites or production of short-chain fatty acids. The underlying mechanisms by which organ-function communicate with gut microbes and the mechanisms by which gut microbiota reciprocates to these host organ signals are largely unknown. In my lecture, experimental data illustrating the interplay between gut microbiota, organ function, especially skeletal muscle and brain development will be presented. I will also touch upon some ideas how we may translate our findings into next generation of food intervention therapies to prolong human health-span.

BIO

Sven Pettersson, MD & PhD, is a cell biologist focusing on microbiome mediated mechanisms regulating mammalian host physiology. Ongoing projects seek to decipher microbiome mediated signalling pathways and metabolites that support cell metabolism relevant to neurons and muscle cells. Sven Pettersson is a strong proponent of the holobiont concept, which considers human beings as a composite of several different microorganisms that together with the mammalian genome collectively determine body, mind and function. He is highly recognized for discovering a link between the microbial communities in our gut and the development and function of the brain. Ongoing work is directed towards the identification of signaling pathways an microbial metabolites contributing ot gut microbe-brain communication.

Integrating multi-omics data to improve understanding of T2D pathogenesis

Xueling SIM

Saw Swee Hock School of Public Health, National University of Singapore and National University Health System, Singapore

ABSTRACT

Type 2 diabetes (T2D) prevalence is increasing worldwide, with highest increase in Asia where earlier age of diagnosis and higher rates of developing complications will have a significant impact on the quality of life and economics burden of T2D. It is known that individuals of East Asian (EAS) ancestry develop T2D at lower body mass index (BMI) compared to individuals of European (EUR) ancestry, likely attributable to higher fat deposition at the abdominal area. Large-scale genetic association studies have now identified nearly 200 genetic signals associated with T2D in EAS individuals, and >500 across trans-ancestries. In one of the largest non-EUR genome-wide meta-analyses in >400,000 EAS individuals from the Asian Genetic of Epidemiology Consortium (AGEN), we recently identified additional 61 new loci associated with T2D, including four new loci associated only in the BMI-adjusted model (NID2, MYOM3/SRSF10, TSN, and GRB10) specific to EAS individuals. While the genetics may contribute to an individual's susceptibility to T2D, non-genetic factors such as obesity, behaviours, and environmental exposures, are the main drivers of T2D. Other downstream omics products of gene transcription, such as metabolite and protein biomarkers, can be influenced by these other environmental variables and can give a more comprehensive biologic readout of these combined contributors to outcomes. Combining genomics, metabolomics, proteomics, we aim to bridge the gap between the genome and diseases to improve understanding of disease pathogenesis.

BIO

My research focuses on using omics techniques to better understand the etiology of cardio-metabolic traits and diseases. Through the generation of multi-omics data from large population-based studies, I use statistical methods to identify omics biomarkers that increase the risk of disease outcomes and related complex traits. The discovery of such biomarkers can aid in the interpretation of biological pathways, highlight differences in risk profiles across populations, and eventually motivate changes in disease prevention, risk prediction, clinical diagnostics and risk stratification practices. My research programme is further described below according to the following themes: a. Discovery of genetic variants associated with cardio-metabolic outcomes and traits in Asia; b. Using Omics association maps to translate genetic loci into biological insights; c. Applications of human genetics to inform clinical care in Singapore populations.

Abstract selected talk

Modelling genetic polycystic kidney disease using human pluripotent stem cell-derived kidney organoids

Meng LIU[1], Chao ZHANG[1], Ximing GONG[1], Tian ZHANG[1], Angelysia CARDILLA[1], Mihir Yogesh NAIK[1], Huamin WANG[1], Yixuan WANG[1], Mingliang FANG[2], Jia Nee FOO[1,3], Yun XIA[1]

[1]Lee Kong Chian School of Medicine, Nanyang Technological University; Singapore; [2]School of Civil and Environmental Engineering, Nanyang Technological University; Singapore; [3] Human Genetics, Genome Institute of Singapore, Singapore

ABSTRACT

Polycystic kidney disease (PKD) is an inherited disorder characterized by progressive expansion of fluid-filled cysts in the kidney. Autosomal dominant polycystic kidney disease (ADPKD) and autosomal recessive polycystic kidney disease (ARPKD) represent the most common forms of PKD. Treatment options are limited due to lack of models to faithfully recapitulate PKD pathophysiology. Herein, we generated a collection of kidney organoids from both PKD patient-derived iPSCs and genetically engineered hPSCs, alongside stress paradigm, to emulate PKD cystogenesis. Cyst formation within PKD kidney organoids exhibited a myriad of structural and functional abnormalities that are typically manifested in PKD patients. Patient iPSC-derived kidney organoids developed tubular cysts in vivo upon engraftment into the sub-renal capsule space of immunocompromised mice. We also performed a small-scale drug screening and identified two candidate drugs that can effectively attenuate cyst formation in both ARPKD and ADPKD kidney organoids. Mechanistic studies revealed that autophagy plays critical roles in safeguarding PKD kidney organoid from cyst formation. The PKD kidney organoid model offers a versatile platform for understanding disease mechanism, as well as for shortlisting drugs with clinical potential.

Singapore National Precision Medicine Program: Intersections with stem cell technologies

Patrick TAN

PRECISE, Singapore

ABSTRACT

The Singapore National Precision Medicine (NPM) program is a 10-year project aimed at developing and mainstreaming precision medicine in Singapore. Currently in Phase II and coordinated by PRECISE, key goals of NPM include establishment of a deeply phenotyped Asian cohort and clinical implementation pilots to test the cost-effectiveness of precision medicine testing in the Singapore healthcare system. In this presentation, I will present how technologies and platforms established for NPM can enhance large-scale stem cell research in Singapore, and how stem cell technologies can be harnessed for developing further insights from NPM data sets. I will also introduce ACTRIS, a sister organization to PRECISE, tasked with developing stem cell platforms across the nation.

BIO

Patrick TAN is Executive Director of PRECISE (Precision Health Research Singapore), coordinating the nation's National Precision Medicine program. He is Executive Director of the Genome Institute of Singapore, Professor at Duke-NUS Medical School Singapore, and Senior Principal Investigator at the Cancer Science Institute of Singapore. During the COVID-19 pandemic, he led one of Singapore's largest COVID-19 testing facilities (Stronghold Diagnostics Labs), an effort which received the Trade and Industry Ministry Borderless Award, the One Public Service Award, and the Public Administration Medal (Silver). He received his B.A. (summa cum laude) from Harvard University and MD PhD degree from Stanford University, where he received the Charles Yanofsky prize for Most Outstanding Graduate Thesis in Physics, Biology or Chemistry. Other awards include the President's Scholarship, Loke Cheng Kim scholarship, Young Scientist Award (A-STAR), Singapore Youth Award (twice), Chen New Investigator Award (Human Genome Organization), President's Science Award, and the Japanese Cancer Association International Award, and AACR Team Sience Award. He is an elected member of the American Society for Clinical Investigation (ASCI), the Bioethics Advisory Committee (BAC), a Board Member of the International Gastric Cancer Association, and on the Board of Reviewing Editors for Science.

SCSS-Dr Susan Lim Award for Outstanding Young Investigator Presentation

Retinal Cell Therapy: the path to vision regeneration

Xinyi SU[1,2,3,4,5]

*[1]Senior Principal Investigator, Divisional Director (IMCB, A*STAR); [2]Asst. Professor, Research Director, Department of Ophthalmology (NUS); [3]Deputy Directory, NUHS Clinician-Scientist Academy (NUHS); [4]Consultant, Department of Ophthalmology (NUH); [5]Clinician-Scientist, Singapore Eye Research Institute (SERI)*

ABSTRACT

The vast majority of end-stage degenerative retinal diseases that cause poor vision and blindness are currently incurable. Stem cell therapy holds promise as a potential treatment for these blinding conditions. A substantial body of pre-clinical research has illuminated mechanisms of retinal cell transplant in the mammalian retina including neural repair via synaptic reconnection and intercellular cytoplasmic materials transfer. However, recent phase I/II retinal stem cell clinical trials have demonstrated safety, albeit with limited efficacy. This talk will cover our work on developing unique hypo-immunogenic stem cell resources, validating them in humanised mouse models and non-human primate disease models. We will also describe our efforts to translate retinal stem cell therapy, in collaboration with industry partners, into an effective treatment for retinal degenerative diseases.

BIO

Xinyi Su, MB BChir PhD, is an ophthalmologist and vitreo-retinal surgeon at the National University Hospital of Singapore. She is the Divisional Director at the Institute of Molecular and Cell Biology (IMCB, A*STAR) and Research Director of the Department of Ophthalmology (NUS). She runs a broad research program in retinal diseases as Senior Principal Investigator of Translational Retinal Therapeutics Lab at IMCB and Clinician-Scientist at the Singapore Eye Research Institute (SERI). Her research in retinal therapeutics has been published in Nature Biomedical Engineering, Advanced Materials, Nature Communications, among others. She received Asia Pacific Vitreo-retinal Society LDP Gold Medal Award in 2021, Eye and Vision Health Distinguished Award in 2021 and Asia Pacific Academy of Ophthalmology SSO Young Ophthalmologist Award for two consecutive years 2019 and 2020, Ten Outstanding Young People Award (2022) and NMRC Clinician-Scientist Award (2022). Xinyi has a career total of >SGD20 million in competitive research grants, and currently leads several translational research programs on retinal cell therapy.

Abstract selected talk

Understanding the molecular signature of mesenchymal stem cell quality

Padmapriya SATHIYANATHAN[1,2,4], Mavis LOMBERAS[3,4], Xiaohua LU[2,4], Jonathan LOH[2,4], Simon M. COOL[2,4], Alexander LEZHAVA[1,4]

*[1]Genome Institute of Singapore, Agency for Science, Technology and Research (A*STAR), Singapore; [2]Institute of Molecular and Cell Biology, Agency for Science, Technology and Research (A*STAR), Singapore; [3]Department of Orthopaedic Surgery, National University of Singapore, Singapore*

ABSTRACT

Therapeutic application of mesenchymal stem cells (MSCs) to repair damaged or diseased tissues has shown promise in a range of late-stage clinical trials. Routinely, MSCs isolated from donors are serially passaged and evaluated by culture-based assays for critical quality attributes (CQA) to determine their quality. Given the extreme donor-to-donor variability in MSC quality and the high cost associated with GMP manufacturing, this becomes a costly screening process that underpins the increased cost of goods. To this end, we compared the molecular signature of donor-derived MSCs of high- and low-growth capacity through PCR arrays, transcriptome and methylation profiling to identify differentially expressed genes as growth potential is known to be indicative of MSC quality. The resultant list of genes found to be associated with scalability was further screened for expression regulation under conditions that alter MSC potency. An independent batch of donor-derived MSCs was then used to validate the shortlisted marker candidates, which demonstrated the correlation of marker expression with CQAs, confirming them as markers of MSC quality. As we build upon this molecular signature of MSC quality, it would facilitate the development of an in-line quality monitoring system during MSC manufacturing and process development for various purposes.

Characterization of stem cells for cellular therapies

Anis LARBI

Medical and Scientific Affairs, Beckman Coulter Life Sciences, France

ABSTRACT

The use of stem cells is investigated in various clinical settings such as regenerative medicine, diabetes, transplantation, neurological disorders and many more. The established hematopoietic stem cell transplantation usually takes the form of a bone-marrow transplantation. To further develop the use of stem cell therapies many factors are to be considered. This includes a better assessment of cell profile and quality. While pluripotent stem cells such as embryonic stem cells and induced pluripotent stem cells (iPSCs), can differentiate into all cell types, multipotent stem cells, such as hematopoietic stem cells and neural stem cells, can differentiate into some lineages. Unipotent stem cells such as muscle stem cells can differentiate into a single cell type. We will review key studies that demonstrated the need for better standards for stem cell profiling. This includes assessing cell count and phenotypes for the evaluation of product quality in clinical research.

BIO

Anis Larbi (PhD) is an immunologist (University of Sherbrooke, Canada) with >15 years of experience in research. His key expertise is in the biology of aging and flow cytometry. His work at the SIgN A*STAR institute focused on the understanding of immunological/biological aging (>200 publications). He is a 2020, 2021 and 2022 Highly Cited Researcher (Clarivate). He joined Beckman Coulter Life Sciences and since 2022 is a Senior Manager of the Medical and Scientific Affairs department.

A learning journey of TCR-T therapy development

Bo Zhu[1], Haiqiang Mai[2], Rui Chen[3], Li Feng Zhang[3], Ling Peng[3], Si Li[3] and Qi-Jing Li[4]

Institute of Molecular and Cell Biology, Singapore

ABSTRACT

The success of CAR-T therapy against B cell malignancy validates the robustness of soliciting antigen-specific T cells for tumor eradication. Engineering T cell antigen specificity with their natural receptors greatly enlarges the target pool for tumor recognition. However, current TCR-T cell treatments failed to mount comparable responses in patients with solid tumor. It is a preeminent clinical need to develop a time-feasible, cost-efficient, and reliable platform for tumor antigen-specific TCR identification. Moreover, a “druggable” TCR or a stable TCR-T cell production protocol is just the first step of a long journey towards the clinical success of cancer treatment. The immunosuppressive tumor microenvironment, the natural or drug-selected tumor antigen heterogeneity, and the persistence deficiency of anti-tumor immunity prohibit effective and durable responses. Focusing on these key challenges, we will have a work-in-progress discussion on our ongoing bench-to-bedside iterations for TCR-T therapy development.

BIO

Qi-Jing Li was trained by Dr. Mark Davis for TCR antigen recognition at Stanford, where he initiated his scientific career as a T cell biologist. Dr. Li’s early research focused on the sensitivity of TCR. He made a series of contributions to determining the minimal subunit, rate-limiting step, and sensitivity controls of TCR activation. In 2008, Dr. Li started up his independent career at Duke focusing on tumor immunology. Besides the basic research, Dr. Li has been continuously translating his expertise in molecular biology and TCR antigen recognition into cancer immunotherapy and clinical immune monitoring. As an academic scientist, Dr. Li has developed new technology platforms for immunogenomics profiling, antigen-specific TCR identification, engineered various cell therapy tools and designed and aided fifteen phase I/II therapeutical trials against various cancers. As a serial entrepreneur, he has co-founded three clinical stage companies to develop CAR-T, TCR-T and TIL platforms.

Latest advances in ischemic and inflammatory disorders from cytopeutics - focus on stroke and aGVHD

Sze-Piaw CHIN

Cytopeutics Sdn. Bhd, Malaysia

ABSTRACT

Mesenchymal stem cells (MSCs) have attracted attention for their immunomodulation property that is achieved through the release of various mediators in response to injury with subsequent tissue regeneration. We have demonstrated the therapeutic potential of Cytopeutics® MSCs in various ischemic and inflammatory disorders such as ischemic stroke, diabetes and acute graft-versus-host disease (aGVHD). Notably, Cytopeutics® MSCs accelerates recovery among acute ischemic stroke patients as early as 6-week post-treatment with significant difference between groups in functional improvement at 3 months and MRI at 12 months showed reduction in median infarct volume. The upfront use of Cytopeutics® MSCs in combination with standard treatment led to faster and sustained complete response with better overall, relapse-free and disease-free survival in aGVHD patients. Such studies have paved the way to exploit the therapeutic use of MSCs to improve the quality and length of life for patients suffering from ischemic and inflammatory disorders.

BIO

Sze Piaw Chin obtained his MBBS from Newcastle UK in 1995 and his MRCP in 1998. He is currently a consultant physician and cardiologist at CMH Specialist Hospital and Honorary Fellow of the Centre for Stem Cell Research, Faculty of Medicine & Health Sciences, Universiti Tunku Abdul Rahman. Dr Chin has served on expert committees for clinical practice guidelines, and several research and registry steering committees for cardiovascular disease and stem cell research. Dr Chin has over 60 publications in international peer-reviewed journals and presented at international medical conferences for the demonstration of clinical anti-inflammatory and immunomodulatory actions of mesenchymal stem cells (MSC) in cardiomyopathy, stroke and diabetes complications and osteoarthritis. Dr Chin was jointly awarded patents from the USA for his pioneering use of MSC treatment for acute stroke, vernal keratoconjunctivitis and diabetes and has been a joint recipient of numerous grants including the MOSTI Technofund.

Abstract selected talk

Secretome derived from hypoxia preconditioned mesenchymal stem cells promote cartilage regeneration and mitigate joint inflammation via extracellular vesicles

Yanmeng YANG[1,2], Yingnan WU[1], Jian Xiong TAN[1, 2], Eng Hin LEE[1,2], Zheng YANG[1,2]

[1]Department of Orthopaedic Surgery, Yong Loo Lin School of Medicine, National University of Singapore, Singapore; [2]Critical Analytics for Manufacturing Personalised-Medicine, Singapore-MIT Alliance for Research and Technology, Singapore

ABSTRACT

Mesenchymal Stem Cells (MSCs) release a plethora of biologically active factors such as cytokines, chemokines, growth factors, and extracellular vesicles (EVs). These paracrine factors have profound effects on tissue engineering, which could become the basis of future MSCs therapies. Hypoxic condition, as the physiologic environment of MSCs, is of a great potential to enhance the therapeutic effect of MSCs paracrine factors. In our study, the paracrine effect of secretome derived from MSCs preconditioned with normoxia and hypoxia was compared through both in vitro functional assays and in vivo rat osteochondral defect models. Specifically, total EVs were separated from the soluble factors to characterize the predominate active components in the hypoxic secretome. We demonstrated that conditioned medium (CM), as well as the corresponding EVs, derived from hypoxia preconditioned MSCs efficiently promoted the repair of critical-sized osteochondral defects and mitigated the joint inflammation in rat osteochondral defect model. HCM, as well as HEV, were validated to significantly enhance chondrocyte proliferation, migration, and matrix deposition, whereas inhibit IL-1β-induced chondrocytes senescence, inflammation, matrix degradation, and pro-inflammatory macrophage activity. Furthermore, multiple functional proteins as well as EV-miRNAs were enriched by hypoxia preconditioning, implicating complex molecular pathways involved in hypoxia MSCs secretome generated cartilage regeneration.

From the skin to the joints: Clinical translation, commercialisation and the future for cell-based regenerative therapies in orthopaedic surgery: where are we headed?

Francis WONG Keng Lin[1,2,3]

[1]Department of Orthopaedic Surgery, Sengkang General Hospital, SingHealth, Singapore; [2]Musculoskeletal Sciences ACP, SingHealth Duke-NUS Academic Medical Centre; [3]Yong Loo Lin School of Medicine, National University of Singapore

ABSTRACT

The use of cell therapy in orthopaedic surgery applications has made significant progress in the pre-clinical stage, and these advancements has been made to even use the paracrine effect of MSCs, such as that of MSC exosomes. However, the pathway to clinical translation is still fraught with significant challenges, be it commercialisation or regulatory hurdles. Till now, Singapore has yet to see a home grown, routine clinical practice stem cell product available in the market, whereas in overseas and in South-East Asia, these products are already being distributed. We look into the available clinical evidence around the world on cell and regenerative therapy for orthopaedic surgery applications, and advancements in translational medicine that has commercialised successfully. Lastly, we explore potential collaborations on how we can bring pre-clinical sciences to the patients and produce world class products for commercialisation in Singapore and the Asia Pacific region.

BIO

Francis Wong Keng Lin graduated as the pioneer resident in the Clinician Scientist Track from NUHS Orthopaedic Surgery Residency Programme, He completed his Masters of Clinical Investigation (MCI), and PhD in NUS, after receiving the National Medical Research Council (NMRC) Research Training Fellowship (RTF). He has investigated the use of Mesenchymal Stem Cells (MSC) exosomes as a cell-free injectable therapy for chondral defects of the knee and has published his translational research in a top-tiered Orthopaedic Surgery journals. He won the Singapore Orthopaedic Association Young Orthopaedic Investigator's Award in 2013 and was the first Orthopaedic Surgeon to win the NMRC New Investigator Grant (NIG), NMRC RTF and recently, the first Orthopaedic Surgeon to be recognised as an established Surgeon-Scientist with his award of the NMRC Transition Award. He is currently the Research Director of Sengkang General Hospital, the youngest candidate till date to assume such a position in Singapore.

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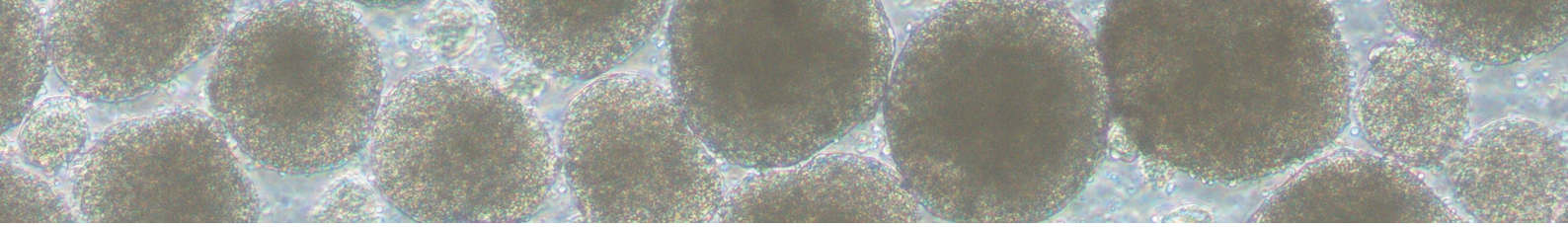

SPEAKER ABSTRACTS

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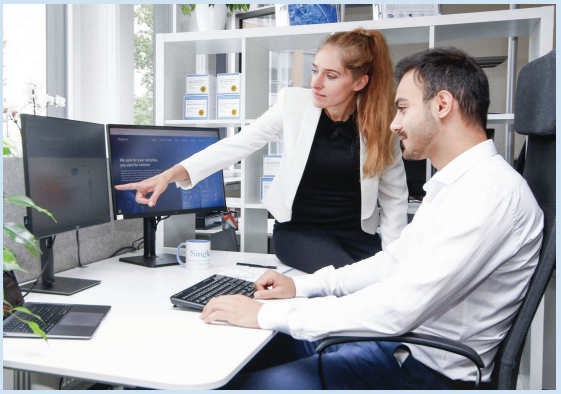

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Label-free biophysical Critical Quality Attributes (CQAs) for cell therapy products

Jongyoon HAN[1, 2, 3,4]

[1]Critical Attributes for Manufacturing Personalized medicine (CAMP) IRG, SMART Centre, CREATE, Singapore; [2]Anti-Microbial Resistance (AMR) IRG, SMART Centre, CREATE, Singapore; [3]Department of Electrical Engineering and Computer Science, Massachusetts Institute of Technology, USA, [4]Department of Biological Engineering, Massachusetts Institute of Technology, USA

ABSTRACT

One of the critical challenges in cell therapy is the lack of reliable, specific, and non-destructive quality attributes, which are sorely needed for all aspects of the biomanufacturing of these cells, including donor selection, in-process quality monitoring, and release testing. Most biological and biochemical assays are destructive or perturbative, which limits their utility, especially for autologous cell therapy. In this talk, I will showcase some of the emerging ideas of label-free, biophysical critical quality attributes (CQAs) we have been developing at SMART CAMP IRG, including electromagnetic and mechanical signatures of cells. Early results show strong correlations with various cellular phenotypes of interest, including cellular senescence and differentiation potential, for which there are no established cell surface markers available currently. These will serve an important role in improving the overall production of both allogeneic and autologous cell therapy products.

BIO

Jongyoon HAN is currently a professor in the Department of Electrical Engineering and Computer Science and the Department of Biological Engineering, at the Massachusetts Institute of Technology. He received B.S.(1992) and M.S.(1994) degree in physics from Seoul National University, Seoul, Korea, and a Ph.D. degree in applied physics from Cornell University in 2001. He was a research scientist at Sandia National Laboratories (Livermore, CA) until he joined the MIT faculty in 2002. He received the NSF CAREER award (2003) and Analytical Chemistry Young Innovator Award (ACS, 2009). His research is mainly focused on applying micro/nanofabrication techniques to a very diverse set of fields and industries, including biosensing, desalination/water purification, biomanufacturing, dentistry, and neuroscience. He is currently the lead PI for MIT's participation in NIIMBL (The National Institute for Innovation in Manufacturing Biopharmaceuticals).

Self-Sustaining Cell-Based Meat Paradigm: Making the muscle cell the serum source

Alfredo FRANCO-OBREGON

National University of Singapore

ABSTRACT

Life, as we know it, evolved in an electromagnetic realm. Indeed, biochemistry itself is set to threshold under weak magnetic fields such as those which naturally envelops the Earth. At the BioIonic Currents Electromagnetic Pulsing Systems (BICEPS) laboratory, our scientific strategy is to exploit this innate biological imperative to promote tissue regeneration and human health. Employing our patented platform technology, we have been able to achieve serum-free growth of muscle cells and are on a path to achieving self-sustaining and GMO- and -drug free muscle growth for ultimate commercial exploitation in the food industry and regenerative medicine. Today, I will discuss our initial achievements at iHealthtech of the National University of Singapore with this platform in the area of cell-based meat production.

BIO

Alfredo FRANCO-OBREGÓN approaches tissue engineering and regeneration from a biophysical perspective, as an alternative to conventional pharmacological interventions. He is particularly interested in how electromagnetic and mechanical forces drive tissue regeneration. Professor Alfredo Franco-Obregón heads the BICEPS (BioIonic Currents Electromagnetic Pulsing Systems) laboratory under the combined auspices of the Department of Surgery and iHealthtech (Institute for Health Innovation & Technology) of the National University of Singapore (NUS) and is actively investigating how magnetic fields promote mitochondrial respiration and downstream developmental and survival adaptations via a process known as Magnetic Mitohormesis. His key areas of interest are skeletal muscle development, stem cell biology and cancer and is a thought leader and innovator in the application of electromagnetics and mechanical forces for tissue engineering and regenerative medicine, clinical applications concerning human health and longevity as well as sustainable food production.

Label-free selection of mesenchymal stem cell subpopulations with more efficacious cartilage regenerative potential

Zheng Yang[1, 2, 3], Lu Yin[3], Yingnan Wu[1, 2], Dahou Yang[3], Eng Hin Lee[1, 2, 3], Jongyoon Han[3, 4]

[1]NUS Tissue Engineering Program, Life Sciences Institute, National University of Singapore; [2]Department of Orthopaedic Surgery, National University of Singapore; [3]Critical Analytics for Manufacturing Personalised-Medicine Interdisciplinary Research Group, Singapore-MIT Alliance in Research and Technology; [4]Department of Electrical Engineering and Computer Science, Biological Engineering, Massachusetts Institute of Technology, USA.

ABSTRACT

Mesenchymal stem cells (MSCs) are potential candidates for cell-based therapies for tissue regeneration. However, the heterogeneity nature of MSCs has significant impact on their therapeutic efficacy. Subjecting MSC to extensive culture expansion is required to generate therapeutically relevant quantities, which lead to heterogenic diversities in cell senescence, differentiation potencies, and secretion of paracrine factors. Therefore, the ability to identify and select an efficacious subpopulation of MSCs targeting specific tissue regeneration holds great clinical significance. We have developed a high-throughput label-free microfluidic technique that separate culture-expanded MSCs into subgroups with distinct size differences and has identified a specific subpopulation with significant proliferation and chondrogenic potency. Delivery of this subpopulation resulted in superior cartilage regeneration in vivo, compared to the hetero-population of MSC. Further, we demonstrated that size-sorted MSC subpopulations within a heterogeneous culture could induce significant impacts to overall population through paracrine signaling. Through repeated microfluidic exclusion of undesirable MSCs subpopulations during continuous culture expansion, selected MSCs with better chondrogenic potential can be generated. This study demonstrates the significant merit of size-based cell selection and provide an effective and practical technology for improved application of MSCs in cartilage regeneration.

BIO

Zheng YANG is a Research Scientist and a Principal Investigator in NUS Tissue Engineering (NUSTEP) Program in the National University of Singapore (NUS). She received her Bachelors of Science (Hons.) and PhD in Biochemistry from Monash University, Australia. She joined NUSTEP in 2005 to further her research in regenerative medicine. As one of the principal Investigator in the Stem Cell & Cartilage Research Group, Yang's research interest is in the investigation and mechanistic understanding of the various microenvironmental cues, and biophysical stimulation, in directing stem cell and chondrocyte cartilage formation, with a focus on developing novel translational treatment to enhance cartilage regeneration. The research has received funding awards from A*STAR Singapore, Singapore National Medical Research Council and the Ministry of Health. She has published over 50 peer-reviewed journal papers, and numerous book chapters, and continues to foster active collaborations with both national and international academic institutions.

Mechanical control of mammalian ovarian folliculogenesis

Arikta BISWAS[1], Boon Heng NG[1], Chii Jou CHAN[1, 2]

[1]Mechanobiology Institute, National University of Singapore; [2]Department of Biological Sciences, National University of Singapore

ABSTRACT

The formation of functional eggs during ovarian folliculogenesis is a critical process in early mammalian development. While genetic studies have revealed key genes for oocyte functions, the underlying mechanisms driving follicle growth remain enigmatic. Recent studies showed that follicle growth is sensitive to mechanical environment, calling for a need to understand mechanical signalling within follicle. Here, we investigate the mechanical functions of theca cells (TCs), which encapsulate the follicles. Using in vivo imaging and ex vivo reconstitution, we demonstrate that the TCs are highly contractile and generate compressive stress to modulate follicle growth. TCs are mechanosensitive to cell stretch and substrate stiffness, suggesting that these cues may in turn regulate TC functions in vivo. We hypothesise that such mechanical feedback is essential for regulating follicle growth and oocyte quality through mechanotransduction pathways. Our work has implications for future understanding and treatment of ovarian disease and infertility.

BIO

I received my PhD degree in 2015 at the University of Cambridge (UK), studying the mechanical and optical properties of living cells and nuclei. From 2016 to 2020, I had my postdoctoral training with Dr. Takashi Hiiragi at EMBL Heidelberg, studying how tissue mechanics and fluid pressure regulate mouse blastocyst size and cell fate specification in early embryo development. I joined the Mechanobiology Institute and the Department of Biological Sciences, National University of Singapore since Jan 2021. My laboratory focuses on understanding how crosstalks between mechanical force and biochemical signaling regulate mammalian ovarian follicle growth during development and ageing. To address these questions, we combine ex vivo reconstitution with quantitative imaging, biophysical, molecular and computational approaches to map out intra-follicular cellular dynamics and mechanical interactions. We are also investigating how reciprocal interactions between the extracellular matrix and macrophages contribute to age-associated inflammation and infertility during ovarian ageing.

Development of mechanotechnologies for high content imaging of scaffolded organoids

Anne BEGHIN[1,2,3], Gianluca GRENCI[1,4], Geetika SAHNI[1], Su GUO[1], Harini RAJENDIRAN[1], Tom DELAIRE[5], Saburnisha Binte Mohamad RAFFI1, Damien BLANC[6], Richard DE METS[1], Hui Ting ONG[1], Xareni GALINDO[5], Anais MONEt[1], Vidhyalakshmi ACHARYA[1], Victor RACINE[6], Florian LEVET[5,7], Remi GALLAND[5], Jean-Baptiste SIBARITA[5], Virgile VIASNOFF[1,8,9]

[1]Mechanobiology Institute, National University of Singapore; [2]Immunology Translational Research Programme, Yong Loo Lin School of Medicine, National University of Singapore; [3]Department of Microbiology and Immunology, National University of Singapore; [4]Biomedical Engineering Department, National University of Singapore; [5]University of Bordeaux, CNRS, Interdisciplinary Institute for Neuroscience, France; [6]QuantaCell, France; [7]University Bordeaux, CNRS, INSERM, Bordeaux Imaging Center, France. [8]Department of Biological Sciences, National University of Singapore;[9]IRL 3639 CNRS, Singapore

ABSTRACT

Current imaging approaches limit the ability to perform multi-scale characterization of three-dimensional (3D) organotypic cultures(organoids) in large numbers. Here, we present an automated multi-scale 3D imaging platform synergizing high-density organoid cultures with rapid and live 3D single-objective light-sheet imaging. It is composed of disposable microfabricated organoid culture chips, termed JeWells, with embedded optical components and a laser beam-steering unit coupled to a commercial inverted microscope. It permits streamlining organoid culture and high-content 3D imaging on a single user-friendly instrument with minimal manipulations and a throughput of 300 organoids per hour. We demonstrate that the large number of 3D stacks that can be collected via our platform allows training deep learning-based algorithms to quantify morphogenetic organizations of organoids at multi-scales, ranging from the subcellular scale to the whole organoid level. We validated the versatility and robustness of our approach on intestine, hepatic, neuroectoderm organoids and oncospheres.

BIO

Virgile VIASNOFF is a Directeur de recherche at CNRS France and an Assoc. Prof at The Mechanobiology Institute of Singapore. He is an expert in mechanobiology. In the past 10 years, his team has been studying the biophysics of plasticity of cell-cell contact and its implication in tissue organization. They developed new technologies to measure, image and control cell-cell junction plasticity in reductionist biological models. They focused on cellular doublets and liver cells. In recent years they extended the scope of their research to spatially inducing cell differentiation in organoids (Gastruloids, hepatic). They study the influence of the spatial organization of stem cell differentiation leading to local (and controllable) patterns of cell-cell adhesion onto the tissue self-organization. They build experimental models and high-throughput imaging techniques to quantitatively connect the mechanobiological response of single cells to the spatial organization of the organoids.

Stem cell differentiation via self-induced physical confinement

Andrew William HOLLE[1, 2]

[1]*Department of Biomedical Engineering, National University of Singapore;* [2]*Mechanobiology Institute, National University of Singapore*

ABSTRACT

Self-induced cellular confinement has been shown to play a role in a wide variety of biological processes. However, the ability of adult stem cells to enter tight confinements has been less studied, despite the fact that stem cells are capable of mechanotransduction, must migrate from their home niche, and possess 'mechanical memory'. Here, we characterize the interstitial spaces in cleared tissue, providing a physiological basis for the design of biomimetic microchannel devices. Next, we show that adipose-derived stem cells (ASCs) and bone marrow stem cells (hMSCs) are capable of entering and permeating PDMS-based microchannels as narrow as 3 μm. Both narrow and wide confinements were shown to induce an upregulation of the osteogenic differentiation marker CBFA1. Interestingly, narrow confinements led to enhanced CBFA1 expression compared to wide channels, suggesting that the level of confinement imposed upon a stem cell via its extracellular environment ultimately plays a role in differentiation.

BIO

Andrew Holle received a Bachelor of Science, Engineering (B.S.E) from Arizona State University, where he worked in the labs of Dr. Christine Pauken and Dr. Deirdre Meldrum. He then received his Ph.D. at the University of California, San Diego, working in Dr. Adam Engler's Stem Cell Bioengineering group. There, he identified the mechanosensitive role of the focal adhesion protein vinculin in substrate stiffness-induced stem cell differentiation. Looking to explore the commonalities between stem cell and cancer mechanobiology, he then joined Prof. Joachim Spatz's Cellular Biophysics group at the Max Planck Institute for Medical Research (Stuttgart, Germany). There, he used photolithography and microfluidics to build microchannel assays to better characterize cancer cell invasion and migration in confinement. His Confinement Mechanobiology lab at the Mechanobiology Institute and in the NUS Biomedical Engineering department focuses on the role of confinement in mechanobiology, with an emphasis on novel strategies for controlling stem cell differentiation.

Efficient generation of functionally relevant hPSC-derived hepatocytes and liver organoids for hepatotoxicity and liver biology modeling

Linda DING[1], Claire HASTON[1], Charis SEGERITZ-WALKO[1], Jennifer CHRISTIE[1], Arthur V. SAMPAIO[1], Ryan K. CONDER[1], Allen C. EAVES[1, 2], Sharon A. LOUIS[1], and Riya SHARMA[1]

[1]Research and Development, STEMCELL Technologies Inc., Canada; [2]Terry Fox Laboratory, BC Cancer, Canada

ABSTRACT

Functionally relevant human hepatocyte models are critical for disease modeling (e.g., NAFLD and NASH), drug safety/efficacy screening, and cell therapy. Human pluripotent stem cell (hPSC)-derived hepatocytes represent a convenient and scalable alternative to current models, which often rapidly de-differentiate in culture or lack metabolic maturity. The STEMdiff™ Hepatocyte Kit supports efficient and reproducible differentiation of hPSCs over 21 days to generate hepatocyte-like cells (HLCs) that exhibit hepatic identity (≥60% ALB+A1AT+ cells) and functionality, including CYP3A4 enzymatic activity and albumin secretion (n = 11-15). Additionally, the HepatiCult™ Organoid Kit (Human) was used to generate passageable HLC-derived liver organoids amenable to cryopreservation and further maturation in 3D, resulting in significant downregulation of the fetal hepatocyte gene, AFP, and upregulation of the mature hepatic genes, ALB and CYP3A4. Both HLCs and HLC-derived organoids exhibited sensitivity to ketoconazole- and rifampicin-induced hepatotoxicity, demonstrating the utility of hPSC-derived liver models for liver research.

BIO

Riya Sharma is the scientific lead of the Liver group in the Research and Development department at STEMCELL Technologies, managing the development of products and workflows to support hepatic research. Some of her group's recent developments include the HepatiCult Organoid Kit for the culture of tissue-derived human liver organoids, and the upcoming STEMdiff Hepatocyte Kit for the generation of functional hepatocyte-like cells from human pluripotent stem cells. Riya completed her studies in Biotechnology at the University of British Columbia, and held research positions in the labs of Dr. Alex Scott developing tendon injury models, and Dr. Paul Rennie screening small molecule therapeutics for the treatment of castration-resistant prostate cancer, prior to joining STEMCELL Technologies in 2016.

Implementing personalised medicine for cystic fibrosis patients: Personalised stem cell derived organoids to select optimal treatment for each patient

Shafagh WATERS

Medicine & Health, University of New South Wales (UNSW), Australia

ABSTRACT

Shafaq Waters' team has developed an Australian national biobank of stem-cell-derived airway and gut organoids, and has built a platform for high-throughput therapy-testing on patients organoids. She combines her unique strengths in organoid disease modelling, multi-omic molecular profiling and computational research with clinical data to improve individualised outcomes for patients with CF. This presentation will discuss the application of the functional drug assessment platform by presenting comprehensive characterisation of multiple ultra-rare CFTR mutations in CF patient derived organoids tested against clinically available and novel CFTR modulating compounds.

BIO

Shafagh Waters (BSc, MSc (Disc.), PhD) is a Scientia senior lecturer at UNSW and an honorary senior scientist at Sydney Children's Hospital. A productive PhD (2012; ANU) and postdoctoral fellowships (2013-2016; UNSW) helped her secure international training fellowships in gene therapy and organoid medicine, establishing her independent lab in 2016. Dr Waters leads an NHMRC funded research program on adult-stem-cell biology for cystic fibrosis (CF) that is supported by international and national industry partnerships. Her work has attracted >35 grants/awards and she was the recipient of a NSW Young Tall Poppy Science Award 2022.

DIVERSITY: A national organoid biobank for translational research

Nimmi BABY

Singapore Translational Cancer Consortium (STCC); Consortium for Clinical Research and Innovation Singapore (CRIS)

ABSTRACT

Singapore Translational Cancer Consortium (STCC) is a business unit under CRIS, MOH. We partner with key stakeholders and institutions to implement joint platforms, bring together key opinion leaders in the cancer research ecosystem and elevate Singapore's competitiveness in cancer research at the global-stage. STCC's Translational Research Integration & Support platform is a one-stop-shop for various advanced molecular assays, providing coordinated support for studies enrolled under STCC. In our next phase, the platform will introduce the DIVERSITY programme - a national effort to synergise research groups in Singapore with organoid expertise and establish high-quality organoid models of Asian-prevalent cancers. Here, our initial efforts in generating tumour organoids of Asian-prevalent cancers will be presented. Further work will involve extensive characterization with various molecular assays, integrated with clinical annotations and drug sensitivity profiles. The biobank will serve as a national resource for pre-clinical research and accelerate Precision Oncology efforts in Singapore.

BIO

Nimmi Baby obtained her PhD and post-doctoral training from the National University of Singapore. She has extensive experience in cellular and molecular biology research. She is also a recipient of the NUS Presidents Graduate Fellowship (PGF) for exceptional accomplishments in research and contributed to several research papers in top international journals. She is currently working as a Project Manager at Singapore Translational Cancer Consortium (STCC), managing and coordinating end-to-end clinical translational research studies.

Modelling neural disorders using human pluripotent stem cells

Shawn JE[1,2]

[1]Molecular Neurophysiology Laboratory, Neuroscience and Behavioral Disorders Program, Duke-NUS Medical School, Singapore; [2]SingHealth Advanced Bioimaging Centre, SingHealth, Singapore

ABSTRACT

The ability to make functional neural cells from human pluripotent stem cells (hPSCs) provides a unique opportunity to study human brain development and neural disorders. In this seminar, I will present recent findings from our laboratory -1) the direct induction and functional maturation of human forebrain glutamatergic and GABAergic neurons derived from hPSCs, 2) the generation of human midbrain-like organoids from hPSCs, and 3) their utilities in modeling human brain disorders.

BIO

Shawn Je is currently an Associate Professor (with tenure) in the Neuroscience and Behavioral Disorders Program at Duke-NUS Medical School in Singapore and a director at SingHealth Advanced Imaging Centre. He received his B.S. from KAIST, M.S. from the University of Michigan, Ann Arbor, and his PhD in Neuroscience and Genetics from the Graduate Partnership Program at the National Institutes of Health (NIH) through the George Washington University Medical School. Then, he pursued postdoctoral training at the Howard Hughes Medical Institute (HHMI) / Duke University Medical School. He joined Duke-NUS Medical School in late 2010 as an assistant professor and received his tenure in 2017. The focus of his research is on the molecular and cellular mechanisms underlying neurological and psychiatric disorders.

Human pluripotent stem cell-derived pancreatic islet cells for human diabetes modelling and target discovery

Natasha Hui Jin NG

Institute of Molecular and Cell Biology, Singapore

ABSTRACT

Since the early efforts to differentiate human pluripotent stem cells (hPSCs) towards pancreatic progenitors and subsequently to islet-like cells, much progress has been made to utilise these cells to investigate the molecular mechanisms underlying diabetes development, including both the rare, monogenic subtype of diabetes, maturity onset diabetes of the young (MODY), as well as the common, chronic type 2 diabetes. Previously, hiPSC-derived pancreatic cells derived from HNF4A-MODY1 and HNF1A-MODY3 patients were found to exhibit specific defects in the regulation of beta cell developmental and functional genes. Further to our disease modelling studies, we sought to map out genomic targets of HNF4A and HNF1A in hPSC-based cell models using ChIP-Seq analysis, to better define the gene network regulated by both transcription factors. Here, I describe current efforts in using hPSC-derived pancreatic islet cells for determining the biology of human HNF4A and HNF1A, and their downstream targets that may play a role in governing insulin secretion from pancreatic beta cells.

BIO

Natasha Ng is a Senior Research Fellow in the Stem Cells and Diabetes Lab (Adrian Teo Lab) at IMCB. She has more than nine years of experience in diabetes research. Her current work seeks to understand the genetic and molecular mechanisms underlying the development of diabetes using stem cell technologies, to uncover new therapeutic pipelines for tackling the disease. She is driving several translational projects and recently co-founded a spin-out, BetaLife, to develop iPSC-based cell therapy for regenerative medicine in diabetes. Natasha previously graduated with a BSc in Biology at Imperial College London, followed by a DPhil in Medical Sciences at the University of Oxford in 2016. Aside from her research, she is additionally co-founder of BioMe Oxford, a UK-based, medical device start-up developing a targeted gastrointestinal sampling device to capture the human microbiome. She is also Advisor of Biotech Connection Singapore (BCS), a non-profit that aims to promote and support life sciences entrepreneurship.

Modular hydrogels for organoid-based disease modelling

Geraldine JOWETT[1,2], Michael D.A. NORMAN[1], Tracy T.L. YU[1], Christian D. LORENZ[3], Joana F. NEVES[2], Eileen GENTLEMAN[1]

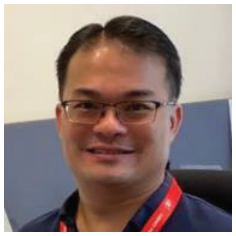
[1]Centre for Craniofacial and Regenerative Biology, King's College London, London, UK; [2]Centre for Host-Microbiome Interactions, King's College London, London, UK; [3]Department of Physics, King's College London, UK

ABSTRACT

Pathological matrix remodelling drives many human diseases, but is challenging to study as in vitro models cannot replicate many complex 3D cell-matrix interactions. To tackle this challenge, we built 3D models of the human gut that allowed us to uncover an unexpected role for a rare immune cell type called ILC1 in driving intestinal fibrosis in patients with Crohn's disease. We used molecular dynamics simulations to design PEG hydrogels that cross-link quickly, but still mimic the stiffness of normal intestinal tissue. We then co-cultured encapsulated human iPSC-derived intestinal organoids with ILC1, and using a combination of atomic force microscopy force spectroscopy and multiple particle tracking microrheology, found that ILC1 drive intestinal matrix remodelling through a balance of MMP9-mediated matrix degradation and TGFβ1-driven fibronectin deposition. Our findings demonstrate the potential of synthetic hydrogels in disease modelling, and open the possibility of unravelling how pathological matrix remodelling contributes to disease.

BIO

Eileen Gentleman is a Reader in the Centre for Craniofacial & Regenerative Biology at King's College London. Eileen joined Imperial College London in 2005 as a post-doctoral research associate (Stevens Group) after completing her PhD in Biomedical Engineering (Tulane University, USA). In 2011, she was awarded a Wellcome Trust Research Career Development Fellowship and moved to King's where her research focuses on developing biomaterials to modulate the physical and biological properties of the 3D cell niche to control stem cell differentiation for tissue engineering and disease modelling. Her work has been published in Nature Materials, Nature Biomedical Engineering, PNAS, and Biomaterials. Eileen has received funding awards from the Wellcome Trust, UK Research and Innovation, and her research in regenerative medicine was recognised with a prestigious Philip Leverhulme Prize. In 2021, Eileen was awarded a Supervisory Excellence Award for her work in mentoring and supporting early career researchers.



Strategies to improve cardiovascular disease modelling in vitro

Boon Seng SOH

Institute of Molecular and Cell Biology, Singapore

ABSTRACT

Cellular derivatives from pluripotent stem cells (PSCs) are currently used to model a myriad of human diseases, supplementing the use of animal models. However, PSC-derived cardiomyocytes (PSC-CMs) are highly heterogenous (nodal, atrial and ventricular cardiomyocytes) and tend to be fetal-like or immature, posing challenges in modelling cardiovascular diseases that mainly affect the aged. In this presentation, I will cover our recent efforts and strategies taken to enhance the maturation of PSC-CMs and the purification of ventricular cardiomyocytes. In addition, as tissue organoids generated from human pluripotent stem cells are valuable tools for disease modelling and understanding developmental processes, my talk will also include the robust generation of human chambered cardiac organoids from pluripotent stem cells for improved modelling of cardiovascular diseases.

BIO

Boon Seng Soh obtained his B.Sc. from the National University of Singapore. He did his PhD thesis on the optimization of hES cell culture and differentiation towards pulmonary stem cells under the A*STAR Graduate Scholarship. In 2011, he joined Prof. Kenneth Chien at Harvard University to work on the biology of multipotent cardiac stem cells, in both murine and human based model systems. He is currently a Principal Investigator at IMCB, A*STAR and is also an Adjunct Assistant Professor at the Department of Biological Sciences, NUS. His research focus has always been clinically driven with emphasis on understanding the underlying molecular and cellular mechanisms in diseases and development of therapies.

Abstract selected talk

Retinal organoid: a model to study embryonic retinal development

Ying CHEN[1,2,3], Yingying ZENG [2,4], Yuin-Han LOH[1,2,3]

*[1]Integrative Sciences and Engineering Programme, National University of Singapore, Singapore; [2]Laboratory for Epigenetics, Stem cells & Cell Therapy, Programme in Stem Cell, Regenerative Medicine and Aging, A*STAR Institute of Molecular and Cell Biology, Singapore; [3]Department of Biological Sciences, National University of Singapore, Singapore; [4]School of Biological Sciences, Nanyang Technological University, Singapore*

ABSTRACT

Over the past decade, retinal organoid differentiation from human pluripotent stem cells has made great advancements. The essential molecular program for retinal development (mostly identified from murine studies) is preserved in retinal organoid differentiation, therefore allowing it to be an excellent in-vitro model to study human embryonic retinogenesis. To further identify factors essential for fate determination during retinogenesis, our team has performed a single-cell RNA sequencing on various timepoints during retinal organoid differentiation. Through pseudo-time analysis, a list of candidates has been identified for retinal ganglion cell lineage. Notably, besides unknown factors, the list also contains known factors, including ATOH7, POU4F2 and ISL1, further supporting the sensitivity of our method.

Kidney organoids for disease models and therapeutic development

Ryuji MORIZANE[1,2,3,4]

[1]Nephrology Division, Massachusetts General Hospital, USA; [2] Department of Medicine, Harvard Medical School, USA; [3]Wyss Institute, Harvard University, USA; [4]Harvard Stem Cell Institute, Harvard University, USA

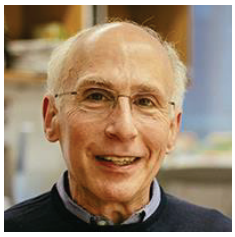
ABSTRACT

We have developed an efficient, chemically defined protocol for differentiating human pluripotent stem cells into multipotent nephron progenitor cells that can form kidney organoids. Kidney organoids contain epithelial nephron-like structures expressing markers of podocytes, proximal tubules, loops of Henle, and distal nephrons in an organized, continuous arrangement that resembles the nephron in vivo. The organoids express genes reflecting various transporters seen in the adult metanephric-derived kidney, enabling the assessment of transporter-mediated drug nephrotoxicity. Repetitive injury to tubular cells causes interstitial fibroblast expansion with characteristics of myofibroblasts, modeling kidney fibrosis in vitro. Organ-on-chip facilitates kidney organoid vascularization and maturation under flow, providing a physiological model of polycystic kidney disease (PKD) for the identification of disease mechanisms and therapeutic candidates. Hence the generated kidney organoids are effective tools to study genetic disorders of the kidney as well as mechanisms of acute and chronic kidney disease.

BIO

Ryuji Morizane is a Principal Investigator at Massachusetts General Hospital, an Assistant Professor at Harvard Medical School, an Affiliated Faculty at Harvard Stem Cell Institute, and a Visiting Scholar at Wyss Institute. He has pioneered research in stem cell differentiation and kidney organoids. He directs research groups focused on kidney regenerative medicine, genome editing in stem cells, and kidney disease modeling. His research has been recognized internationally, and he has received various awards including NIH Director's New Innovator Award.

KEYNOTE LECTURE



De-aging the aging brain

Lee L. RUBIN

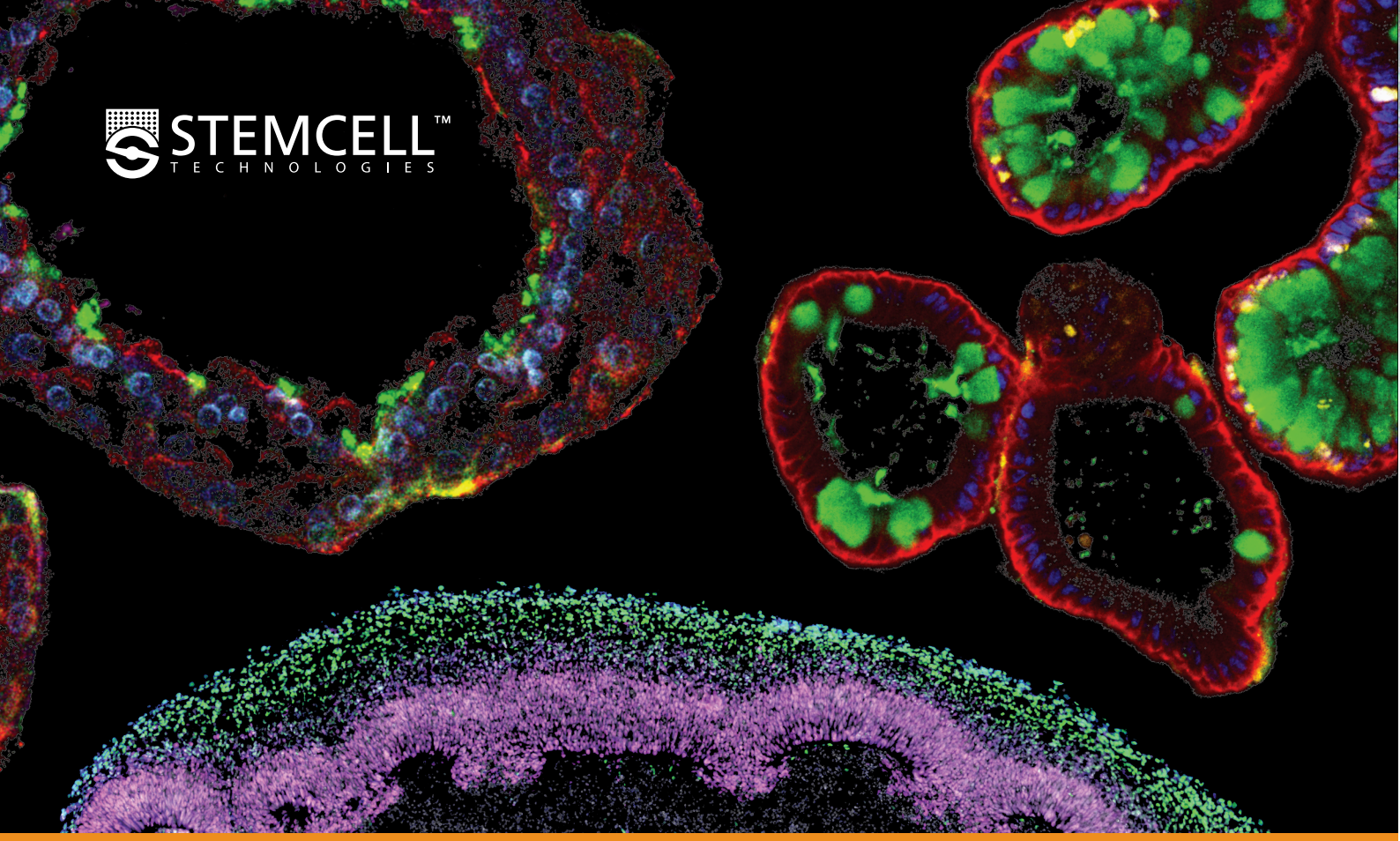
Dept of Stem Cell and Regenerative Biology, Harvard University and the Harvard Stem Cell Institute, Cambridge, MA, USA

ABSTRACT

Cognitive function declines with normal aging and, in an accelerated fashion, with a variety of neurodegenerative diseases. To date, few, if any, therapeutics have shown a clear ability to slow this decline. Yet, experiments over the past two decades have uncovered perturbations – exercise being the most famous – that exert a positive effect on brain function. Furthermore, over the past several years, another set of studies, originally employing a classical method known as parabiosis, have revealed the brain as one of the most responsive tissues to fluctuations in the levels of blood-borne factors. In fact, convincing data have shown that aspects of aging can be reversed by exposure to each of more than 10 factors. Our work has focused on changes in brain vasculature that follow treatment with GDF11 and other factors and the improvement in neural function that is driven by this “rejuvenated” vasculature.

BIO

Lee Rubin is Professor of Stem Cell and Regenerative Biology at Harvard University and Co-Director of the Neuroscience Program at the Harvard Stem Cell Institute. His work focuses on neurodegenerative and neuromuscular disorders and has a strong translational focus. Currently, his efforts are focused on producing muscle stem cells to treat muscular and neuromuscular disorders and, importantly, on discovering therapeutics capable of reversing the degenerative changes associated with brain aging.



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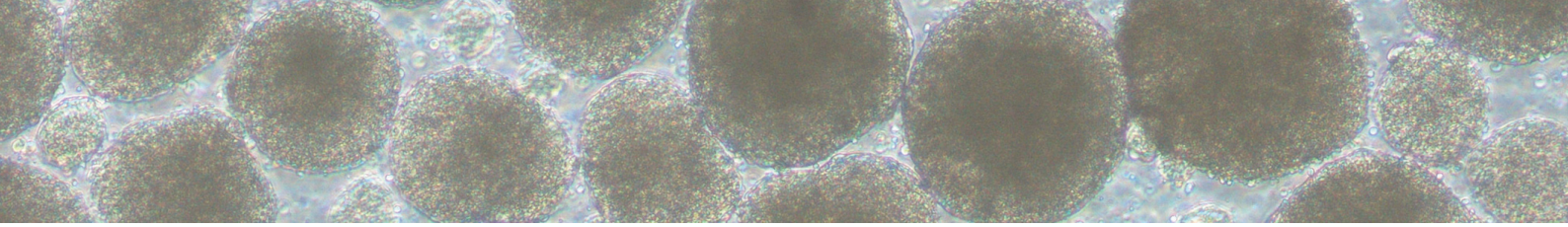
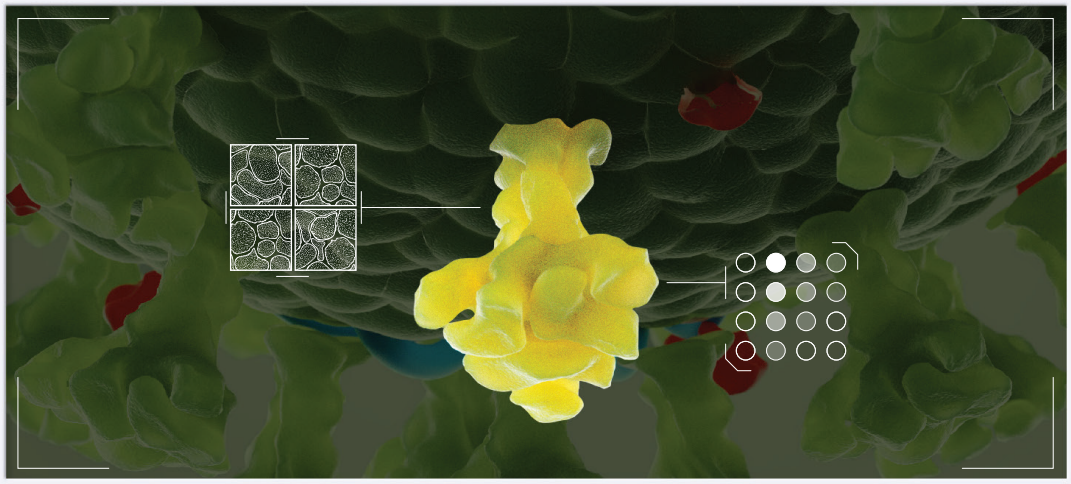
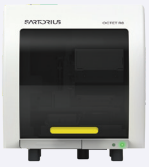


POSTER

ABSTRACTS





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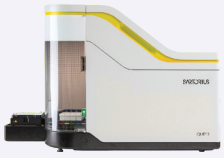

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SARTORIUS

P1

Osteoclast progenitor recruitment and differentiation is controlled by osteogenic Cxcl9

Quang Tien Phan[1], Wen Hui Tan[1], Kevin Yiqiang Chua[2], Aizhen Jin[3], Woon-Puay Koh[3,4], Christoph Winkler[1]

*[1]Department of Biological Sciences and Centre for Bioimaging Sciences, National University of Singapore, Singapore 117543, Singapore' [2]Integrative Sciences and Engineering Programme, NUS Graduate School, National University of Singapore, Singapore; [3]Healthy Longevity Translational Research Programme, Yong Loo Lin School of Medicine, National University of Singapore, Singapore; [4]Singapore Institute for Clinical Sciences, Agency for Science Technology and Research (A*STAR), Singapore*

In osteoporosis, an excessive formation of osteoclasts with abundant bone-resorbing activity is responsible for bone erosion and potential fractures. Although osteoclasts are known from cell culture experiments to form from the bone marrow-derived macrophage lineage, the detailed mechanisms underlying macrophage recruitment and differentiation into osteoclasts *in vivo* are poorly understood. Using a transgenic medaka fish osteoporosis model where ectopic osteoclast formation and osteoporotic lesions are induced by transgenic Rankl expression, we showed that bone-forming osteoblast progenitors strongly express Cxcl9 upon Rankl induction. This in turn triggers the recruitment of a subset of Cxcr3.2-expressing macrophages towards the bone surface. There, macrophages dynamically interact with osteoblast progenitors and start differentiating into Cathepsin K-positive osteoclasts, which resorb bone and cause severe bone lesions in the vertebral column. Blocking Cxcl9-Cxcr3.2 signaling in medaka by CRISPR-induced mutations or treatment with chemical CXCR3 antagonists prevents macrophage recruitment and osteoclast formation, and protects ossified tissues from Rankl-induced bone loss. To further investigate whether these findings made in medaka are relevant to human bone erosion-related diseases, we examined CXCL9 levels in sera of patients with incident hip fractures and compared them to control subjects who did not experience fractures. For this, serum samples had been collected on average 6.3 years before the hip fracture had happened. We observed significantly higher CXCL9 levels in pre-facture sera of men when compared to the non-fracture matched-control subjects. No such difference was seen in women. These results suggest that CXCL9 could potentially be used as a marker to predict osteoporotic hip fractures in men and that targeting CXCL9-CXCR3 signaling may be a novel therapeutic avenue to prevent and treat osteoporosis.

P2

Nanopore tomography fingerprinting of viral particles in cell therapeutics

Kun Li[1], Arjav Shah[2], Patrick Doyle[1,2], Slaven Garaj[1,3]

[1]Singapore-MIT Alliance for Research and Technology Centre, CREATE Tower, Singapore; [2]Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts, 02139, USA; [3]Department of Physics, National University of Singapore, Singapore

Safety testing (quality control) is critical process – and a bottleneck – in the cell therapy workflow. There is an urgent need to develop a rapid, highly sensitive and specific, broad-spectrum, low-cost, and non-destructive method for the adventitious agent detection in cell products, especially for infectious viral contaminations. Here we present our progress in the development of a virus fingerprinting platform based on solid state nanopores, and associated methodology that does not require PCR, labelling, or cell-culturing. The nanopore tomography (NT) relies on capturing individual viral particles with a solid-state nanopore using electrophoretic trapping. As the particles translocate, it modulates the flow if ions through the nanopores (current blockade, ΔI), in a particular pattern that is indicative of the particle's geometry and surface chemistry. We measured nanopore tomography of various objects, such as dsDNA, phage, polystyrene particles, DNA nanoparticles, small vesicles (exosomes), human simplex virus (HSV), and adenovirus. Our results demonstrate a clear discrimination between ΔI signal from those particles, based on their size, shape, surface charge, and surface structure. We are further developing fine-grained discrimination between various viruses, developing the library of ΔI signature, and employing machine learning approaches to achieve the automated virus detection and the real-time monitoring of cell culture supernatants.

P3

High density CAR T cell culture with a 2-ml continuous perfusion bioreactor

Wei-Xiang SIN[1], Denise Teo[1], Kevin S. Lee[2], Harry Lee[2], Narendra Suhas Jagannathan[3], Rajeev Ram[1,4], Lisa Tucker-Kellogg[1,3], Michael Birnbaum[1,4]

[1]Critical Analytics for Manufacturing Personalized-Medicine (CAMP), Singapore-MIT Alliance for Research and Technology (SMART), Singapore; [2]Erbi Biosystems Inc., USA; [3]Duke-NUS Medical School, Singapore; [4]Massachusetts Institute of Technology (MIT), USA

With six CAR T cell therapy products currently approved by the US FDA for the treatment of hematological cancers, there are increasing efforts to develop novel and better manufacturing technologies and processes to improve efficacy, reduce variability, and reduce cost. However, due to limited or costly materials, such as human PBMCs and viral vectors, research- and preclinical-scale experiments in cell therapy are often done in milliliter-sized static culture with minimal environmental control, which can result in variabilities that may prove difficult to translate to larger culture systems. We leveraged an automated perfusion microbioreactor, which can replicate bench-scale perfusion processes, to generate functional CAR T cells at high cell densities of >100e6 cells/mL in a small 2-mL culture volume. We performed in-place activation, transduction, and expansion within the microfluidic bioreactor, and showed that with improved media exchange from continuous perfusion, cell expansion is improved, achieving nearly patient dose levels with >400-fold expansion over a 14-day culture, from 6e5 cells to >200e6 cells, at consistently high cell viabilities of >95%. These proof-of-concept data demonstrate the utility of the system as both a process development tool as well as a potential future manufacturing platform.

P4

Novel anti-microbial peptides derived from differentiating adipose-derived stem cells against chronic wound infection

Smarajit Chakraborty[1], Vikashini Ravikumar[1], Brian Chia[2], Kamaladasan Kalidasan[3] Shigeki Sugii[1]

*[1]Cell Biology and Therapies Division, IMCB, A*STAR, Singapore; [2]Experimental Therapeutics Centre, A*STAR, Singapore; [3]A*STAR Skin Research Labs (A*SRL), Singapore*

Chronic wound is a large and growing global problem predominantly due to the emergence of multidrug and antibiotic resistant (MDR) bacterial pathogens, particularly in Singapore challenged with increasing occurrence of aging population and diabetes mellitus. Adipose-derived mesenchymal stem cells (ASCs) are shown to migrate to the wound sites, wherein they encounter invading pathogenic bacteria, a niche that is insufficiently studied, yet critical in determining the fate of the wound progression. We successfully developed a unique co-culture setup between bacteria and ASCs and discovered robust antibacterial properties of conditioned media (CM) derived from intermediate adipogenic differentiating ASCs triggered by bacteria priming, which has never been reported before to our knowledge. Activated ASC-CM exhibited robust bacterial killing, significant antibiofilm effects against wound infecting pathogens, accelerated wound healing, and prevented antimicrobial resistance (AMR). Through proteomics analysis and confirmatory experiments, we have identified ultra-short regions (>21-residues) within potent, non-toxic, novel antimicrobial peptides (AMPs) which exhibited enhanced antimicrobial efficacy, prevented acquisition of AMR, improved migration of human keratinocytes, exhibited reduced cytotoxicity and hemolysis, compared to AMP, pexiganan (Phase III clinical trial) and antibiotics. We aim to develop our lead preclinical peptide into a clinical candidate for the treatment of chronic wound infections.

P5

Magnetic Resonance Relaxometry as a tool for tracking induced pluripotent stem cell variability

Daniel Roxby[1,2], Tan Dai Nguyen[1,2], Jerome Tan[1,2], Jiahui Chen[1], Wai Hon Chooi[3], Shi-Yan Ng[3], Sing Yian Chew[1,2,4], Jongyoon Han[2,5,6]

*[1]School of Chemistry, Chemical Engineering and Biotechnology & Lee Kong Chian, School of Medicine, Nanyang Technological University, Singapore; [2]CAMP IRG, SMART Centre, CREATE, Singapore; [3]Institute of Molecular and Cell Biology, A*STAR; [4]Singapore, School of Material Science and Engineering, Nanyang Technological University, Singapore; [5]Department of Biological Engineering, Massachusetts Institute of Technology, Massachusetts, USA; [6]Department of Electrical Engineering and Computer Science, Massachusetts Institute of Technology, Massachusetts, USA*

In the development of cell therapy, iPSCs have significant advantages, specifically, their pluripotency, donor matching capabilities and self-renewal. From the laboratory to a manufacturing setting, self-renewal, the ability to perpetually proliferate, passage after passage is especially useful. Yet, it is widely observed that iPSCs produced from culture can be subject to significant quality variations, caused by known factors (handling protocols, passage numbers, etc.) and uncontrollable factors. Currently, no method exists to quickly establish, in a label-free manner, if one passage of iPSCs is the same as prior, nor the initial passage. In this study, we introduce Magnetic Resonance Relaxometry (MRR) for the quick, label free measurement of the quality, variability, and pluripotency of iPSC cultures. This method uses just 100k cells for measurements that take 2 minutes with no chemical or biological processing. T2 relaxation time from MRR is strongly correlated with the intracellular iron (Fe3+) content. We demonstrated that several culture factors contribute to variations in resulting iPSCs, and that T2 measurement can identify such variations. While it is desirable to control these variations and processes tightly, we believe that MRR is a useful tool in quantifying iPSC quality and variability commonly observed in lab or manufacturing settings.

P6

Label-free assessment of differentiation efficiency in iPSC-derived Spinal Cord Progenitor Cells via Magnetic Resonance Relaxometry (MRR)

Jerome Tan Zu Yao[1,2], Jiahui Chen[1], Daniel Roxby[1,2], Wai Hon Chooi[3], Tan Dai Nguyen[2], Ng Shi Yan[3], Sing Yian Chew[1,2,4], Jongyoon Han[2,5,6]

*[1]School of Chemistry, Chemical Engineering and Biotechnology & Lee Kong Chian, School of Medicine, Nanyang Technological University, Singapore; [2]CAMP IRG, SMART Centre, CREATE, Singapore; [3]Institute of Molecular and Cell Biology, A*STAR, Singapore, [4]School of Material Science and Engineering, Nanyang Technological University, Singapore; [5]Department of Biological Engineering, Massachusetts Institute of Technology, Massachusetts, United States of America; [6]Department of Electrical Engineering and Computer Science, Massachusetts Institute of Technology, Massachusetts, USA*

The advent of induced pluripotent stem cells (iPSC) has provided a promising solution to the replacement of damaged neurons, especially in spinal cord injuries. Despite its merits, differentiation of iPSCs is a highly variable process, prompting the need to reliably assess the degree of differentiation across batches, and validate their quality. iPSC phenotypes are detected through labelling cells with fluorescent markers or immunofluorescence staining based methods, which perturb or destroy cells, preventing their further use. In this study, human iPSCs derived from Cord Lining Endothelial cells were differentiated into Spinal-cord Progenitor Cells (SCPCs) through a 10-day process. Label-free measurement of these cells were performed at different timepoints using Magnetic Resonance Relaxometry (MRR), a rapid and label-free technique to obtain critical cellular iron (Fe3+) content. MRR only requires <180k cells for measurements that takes up to 2 minutes without additional preparation. SCPCs have significantly different T2 relaxation times compared to iPSCs. Furthermore, SCPCs harvested at the end of the differentiation containing higher levels of residual pluripotent markers have lower T2 relaxation times when compared to SCPCs with lower levels of these markers. Our technology provides an efficient, label-free method to assess critical quality attributes of iPSCs and SCPCs.

P7

Multi-dimensional double spiral microfluidic selection of MSC subpopulation with more efficacious chondrogenic potential

Zheng Yang[1,2,3], Dahou Yang[1], Yingnan Wu[2,3], Vinitha Denslin[3], Jaylen Tan[1], Eng Hin Lee[1,2,3], Jongyoon Han[1,4]

[1]Critical Analytics for Manufacturing Personalised-Medicine Interdisciplinary Research Group, Singapore-MIT Alliance in Research and Technology, Singapore; [2]Department of Orthopaedic Surgery, National University of Singapore, Singapore; [3]NUS Tissue Engineering Program, Life Sciences Institute, National University of Singapore, Singapore; [4]Department of Electrical Engineering and Computer Science, Biological Engineering, Massachusetts Institute of Technology, USA

Despite increasing clinical application of MSCs in cartilage regeneration, the outcomes have been plagued by substantial variability and inconsistency. A large contributor to such unpredictable outcomes stemmed from the heterogeneity nature of MSC. The heterogeneity stem first from the inherent donor-to-donor variant in MSC functionality, that is influent by the genetic, age and health conditions of the donor. During laboratory propagation to generate critical cell quantity for therapeutic application, processing and protocol differences further perpetuates cell heterogeneity, critically affecting the functional quality of the expanded cells. There is thus a growing need to manage the heterogeneity of MSCs in order to improve stem

therapeutic efficacy. In this study, we describe the use of the multi-dimensional double spiral (MDDS) microfluidic device for the segregation of MSC to different size subpopulations, and the identification of a particular size range of MSC with superior chondrogenic potential based on the extent of cartilaginous matrix formation and correlation to magnetic resonance relaxometry (MRR) T2 measurement. Implantation of this MSC subpopulation resulted in significantly improved cartilage regeneration in a rodent osteochondral defect model.

P8

Mesenchymal stem cell enrichment augmented through microfluidics from bone marrow aspirate

Nicholas Tan[1,2], Kerwin Kwek[1], Quek Kai Yun[1], Teo Kim Leng[4], Loberas Mavis Gayle Lumawag[5], Steve Oh[4], Hou Han Wei[1,2,3], Jongyoon Han[1,6]

*[1]Critical Analytics for Manufacturing of Personalized Medicine, Singapore−MIT Alliance for Research and Technology, Singapore; [2]School of Mechanical and Aerospace Engineering, Nanyang Technological University, Singapore; [3]Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore; [4]Bioprocessing Technology Institute, A*STAR, Singapore; [5]NUS Tissue Engineering Programme (NUSTEP), National University of Singapore, Singapore; [6]Department of Electrical Engineering and Computer Science, Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA*

The growing interest in regenerative medicine has opened new avenues for novel cell therapies using stem cells-rich bone marrow samples. Bone Marrow Aspirate (BMA) is an important source of biological sample as it comprises of the highest source of stromal Mesenchymal Stem Cells (MSCs) used for autologous stem cell therapies. However, these cells are extremely rare (0.001% of total nucleated cells). Conventional harvesting methods using centrifugation often lead to poor yield with long processing time, thus advocating a need for novel BMA sample preparation technologies. Microfluidics is an emerging scientific field that has generated substantial advancements in biomedical processes such as cell separation. We aim to exploit the Deterministic Lateral Displacement (DLD) technique, a passive size-based label free cell sorting method in microfluidics, to isolate MSCs from BMA with improved yield and efficiency. The DLD device used to process BMA does not require any sample dilution or preparation. Our results showed that DLD can achieve a better yield with low processing time as compared to centrifugation. To improve throughput scalability, we also developed a novel multiplexed DLD device. Taken together, the DLD platform is highly useful for fast and efficient isolation of MSCs from BMA for effective downstream cell therapy.

P9

Rapid sterility testing of cell therapy products using a multiplex digital Loop-mediated isothermal amplification

Joshua Raymond[1], Xiaolin Wu[1,*], Yaoping Liu [1,2], Jongyoon Han[1,2,3], Timothy Lu[1,2,3], Hanry Yu [1,4]

*[1]SMART-CAMP, Singapore; [2] SMART-AMR, Singapore; [3]Biological Engineering, MIT, United States; [4]MECHANOBIOLOGY, NUS, Singapore; *Corresponding author*

It is challenging to have an accurate, rapid and sensitive sterility test for cellular therapy products. The current gold standard as regulated by the United States Pharmacopeia USP <71> are culture based methods for detection of contamination, which requires an incubation of up to 14 days before the sample can be deemed as sterile or contaminated. This 14-day sterility test does not match the urgent medical need for patients and the short shelf life of the cell products. To address this problem, our work developed a sterility test method by combining electrostatic microfiltration and digital Loop-mediated isothermal amplification (dLAMP). For the proof of concept, we have selected four out of the six listed microorganisms in the USP <71> *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Candida albicans* as multiplex detection targets. Using this method, we have been able to concentrate the microorganisms after several hours' incubation in a growth promotion medium. Subsequently we are able to identify the presence of live or dead microorganisms specifically and accurately within seven hours from as low 30 CFU of each organism present in the original sample using dLAMP.

P10

Emergent patterns in mesenchymal stromal cell monolayer predict efficacy of cartilage formation

Ekta Makhija[1], Yang Zheng[1,2,3], Jiahao Wang[4],Han Ren Leong[1,3], Rashidah Binte Othman[1], Ee Xien Ng[1], Eng Hin Lee[1,2,3], Lisa Tucker-Kellogg[1,5], Yie Hou Lee[1,6], Hanry Yu[1,3,4,7], Zhiyong Poon[1,6,8], Krystyn J. Van Vliet[1,9]

[1]SMART CAMP, Singapore; [2]NuSTEP, Singapore; [3]NUS, Singapore; [4]MBI, Singapore; [5]DukeNUS, Singapore; [6]SingHealth Duke-NUS, Singapore; [7]IBN ASTAR, Singapore; [8]SGH, Singapore; [9]MIT, USA

Mesenchymal stromal cell (MSC) therapy is a promising treatment option for repairing damaged cartilage in osteoarthritis. Currently, there is a need to find cell attributes that are early predictors of cartilage repair potential of a batch of MSCs, as the conventional efficacy assay comprises of 3-week long chondrogenic differentiation process. Towards this, we hypothesized that the cellular swirl patterns that emerge in high density cultures of MSCs would correlate with the chondrogenic differentiation outcome, as we observed that certain defect sites within the liquid crystal-like swirl pattern corresponded to mesenchymal condensations – the early stage of cartilage formation. We also observed that the swirl patterns made by cells obtained from different donors were cell batch-specific and reproducible across technical replicates. To explore the possibility of using swirl pattern to predict the chondrogenic differentiation potential of MSCs, we quantified the patterns as 'variance of coherency' (VoC) from actin images of cellular swirls generated across 5 MSC donors. We observed that the day 9 VoC correlated strongly with the cartilage matrix proteins ($r > 0.88$) quantified from the conventional in-vitro chondrogenic differentiation assay. Hence, cellular swirl pattern quantification offers a powerful tool to predict the differentiation outcome of a batch of stem cells.

P11

Detection of microbial contaminations using nicotinic acid to nicotinamide ratio as a critical quality attribute to determine sterility in cell therapy products

Alice Ng[1], Jiayi Huang[1,*], Liang Cui[2], Joshua Raymond[1], Meenubharathi Natarajan[1], Yie Hou Lee[1], Stacy L. Springs[1,3]

[1]Critical Analytics for Manufacturing Personalised-medicine Interdisciplinary Research Group, Singapore-MIT Alliance for Research and Technology Centre Singapore; [2]Antimicrobial Resistance Interdisciplinary Research Group, Singapore-MIT Alliance for Research and Technology Centre, Singapore; [3]Center for Biomedical Innovation, Massachusetts Institute of Technology, USA; [*]Present address: Roquette Singapore Pte Ltd, Singapore

Stem cell therapy is an emerging therapeutic approach with the potential to treat wide range of previously intractable illnesses and medical conditions. Current challenges in cell therapy manufacturing related to the long duration of product sterility tests impede the availability of cell therapies. We have identified the nicotinic acid (NA) to nicotinamide (NAM) ratio as a non-invasive critical quality attribute (CQA) that detects a broad spectrum of microbial contaminants in cell cultures. Importantly, only live microorganisms caused increases in this ratio. Mesenchymal stromal cell (MSC) was used as a cell therapy product (CTP) and liquid chromatography mass spectrometry (LC-MS) was employed to detect presence of NA in bacteria-contaminated CTPs. We established analytical parameters of this method. Various colony-forming unit of E.coli K12 was added into MSC and we found that NA was uniquely present with a significantly higher concentration in these bacteria-contaminated CTPs as compared to uncontaminated MSC. This is due to the conversion from NAM by microbial nicotinamidases, which mammals lack. In summary, NA to NAM ratio is a potential CQA for detection of microbial contaminations in CTPs. We are in the process of translating into a process analytical technology (PAT).

P12

Ribosomal proteins regulate 2-Cell stage transcriptome in mouse embryonic stem cell

Yao Yi[1,2], Yingying Zeng[1,2], Yuin-Han Loh[1]

*[1]Cell Fate Engineering and Therapeutics Lab, Cell Biology & Therapies Division, Institute of Molecular and Cell Biology, A*STAR, Singapore; [2]These authors contributed equally*

A rare sub-population in mouse embryonic stem cells (mESCs), the 2-Cell like cells, is defined by the expression of MERVL and 2-Cell stage specific transcript (2C transcript), which are representative of a stage of expanded pluripotency. Here, we report that the ribosomal proteins (RP), besides their fundamental roles in translation, maintain the identity of mouse embryonic stem cells (mESC) and regulate the expression of 2C transcripts. Dis-regulation of the RP induces expression of these transcripts and alters the chromatin landscape. Mechanically, RP KD impairs TRIM28 binding on *Dux* and drives regulation of *Dux*-dependent early-stage embryonic transcripts via a unique RPL11-MDM2-P53-DUX axis. Specifically, RPL11 binds and inhibits MDM2 function upon RP KD, thus preventing P53 protein degradation and activating its downstream pathways, including *Dux*. Our study delineates the critical roles of RP in 2C transcript activation, ascribing a novel function to these essential proteins.

P13

D-TF1 governs mouse expanded potency and facilities breaking of embryonic lineage barrier

Zi Hao Zheng[1,2,12], Yingying Zeng[1,3,12], Ka Wai Wong[1], Nadia Omega Cipta[1,4], Javier Tan[1], Edison Tay[1], Yih-Cherng Liou[4,5], Justin Jang Hann Chu[6,7,8], Hu Li[9], Derrick Sek Tong Ong[2,10*], Yuin-Han Loh[1,2,4,11*]

*[1]Cell Fate Engineering and Therapeutics Lab, Cell Biology and Therapies Division, ASTAR Institute of Molecular and Cell Biology, Singapore; [2]Department of Physiology, NUS Yong Loo Lin School of Medicine, Singapore; [3]School of Biological Sciences, Nanyang Technological University, Singapore; [4]Department of Biological Sciences, National University of Singapore, Singapore; [5]Proteomics Group, Bioprocessing Technology Institute, A*STAR, Singapore; [6]Department of Microbiology and Immunology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore; [7]Infectious Disease Translational Research Programme, Yong Loo Lin School of Medicine, National University of Singapore, Singapore; [8]Collaborative and Translation Unit for HFMD, Institute of Molecular and Cell Biology, Agency for Science, Technology and Research, Singapore; [9]Center for Individualized Medicine, Department of Molecular Pharmacology & Experimental Therapeutics, Mayo Clinic, Rochester, USA; [10]NUS Center for Cancer Research, Yong Loo Lin School of Medicine, National University of Singapore, Singapore; [11]NUS Graduate School for Integrative Sciences and Engineering, National University of Singapore, Singapore; [12]These authors contributed equally to this work; *Correspondence*

Formation of blastocyst-like structure in vitro using mouse stem cells is restricted to Expanded or Extended Potential or stem cells (EPSC) and 2 Cell-like cells (2CLC). Using multi-omic approaches in combination with a blastoid model, our study investigated genes that control expanded potency in Expanded Potential Stem Cells (EPSC) in a systematic manner. Through comparative analysis of transcriptome and epigenetic profile of EPSC and mouse embryonic stem cells (ESCs), we identify D-TF1 as a key regulator in EPSC. Additionally, we identify another potential EPSC regulator MyD through our D-TF1 ChIP-seq analysis. Interestingly, overexpressing transcription factor D-TF1 and epigenetic regulator MyD can effectively expands cell fate of ESC, allowing the cells to form blastoid in vitro. Taken together, our data suggests a novel mechanism in expansion of pluripotency of stem cells through D-TF1 , allowing formation of self-organised blastocyst-like structure using ESC through single gene overexpression.

P14

Developing physiologically relevant 2D and 3D *in vitro* inherited retinal disease models: To study and develop therapeutics

Animesh Banerjee[1], Munirah Santosa[1], Bhav H Parikh[1], Zengping Liu[2], Kim Chi Tran[2], Queenie T S Woon[1], Xinyi Su[1,2,3,4]

*[1]Institute of Molecular and Cell Biology, A*STAR, Singapore; 2Department of Ophthalmology, Yong Loo Lin School of Medicine (YLL SoM), National University of Singapore (NUS), Singapore; [3]Singapore Eye Research Institute, Singapore; [4]Department of Ophthalmology, National University Hospital, Singapore*

The growing studies focusing on genetic approaches to reverse the inherited retinal disease phenotype demand physiologically relevant in vitro models capable of delivering translatable functional readouts to fast track their screening and hence advancement. To fulfill this unmet need, we aim to develop 2D and 3D in vitro models using the cells derived from Stargardt patients with the ABCA4 mutation. We differentiated hESCs and iPSCs reprogrammed from patient PBMCs into 2D retinal pigment epithelial (RPE) and retinal progenitor (RPCs) cells and 3D retinal organoids (ROs). The retinal cells generated in 2D and 3D contain characteristic retinal cell features assayed via immunofluorescence and the TEER assay (RPEs). The ROs demonstrated brush border-like photoreceptor outer segments with principal retinal cells. To develop a disease-specific functional readout assay, we exposed the RPE cells to blue light stress to amplify the disease-related dysfunction. The mutant RPEs exhibited reduced viability and increased oxidative stress upon blue light exposure, despite maintaining their cellular morphology and electrophysiological properties similar to the control. Together, we develop a 2D and 3D retinal disease model, taking ABCA4 as an example that can be extended to other IRDs. At the same time, disease-specific stress assays can be developed to measure the therapeutic's functional effectiveness in disease recovery.

P15

Genome-Wide CRISPR screen identifies an NF2-adherens junction mechanistic dependency for cardiac lineage

Chang Jie Mick Lee[1,2], Matias I. Autio[1,2], Wen Hao Zheng[1,2], Yoohyun Song[3,5], Shyi Chyi Wang[3,5], Darren Chen Pei Wong[3,4], Wan Kee Chock[1], Boon Chuan Low[3,4,7], Marius Sudol[6], *Roger S-Y Foo[1,2]

*[1]Genome Institute of Singapore, Singapore; [2]Cardiovascular Disease Translational Research Programme, National University Health System, Centre for Translational Medicine, Singapore; 3Mechanobiology Institute Singapore, National University of Singapore, Singapore; [4]Department of Biological Sciences, National University of Singapore, Singapore; [5]Institute of Bioengineering and Bioimaging, A*STAR, Singapore; [6]Icahn School of Medicine at Mount Sinai, Department of Medicine, USA; [7]University Scholars Programme, National University of Singapore, Singapore*

Cardiac differentiation involves a stepwise clearance of repressors and fate-restricting regulators through the modulation of BMP/Wnt-signaling pathways. However, the mechanisms and how regulatory roadblocks are removed with specific developmental signaling pathways remain unclear. Here, we performed a genome-wide CRISPR screen to uncover essential regulators of cardiomyocyte specification in human embryonic stem cells (hESCs) to better delineate the molecular events that control the earliest step of cardiovascular specification. We identified *NF2*, a Moesin-Ezrin-Radixin Like (MERLIN) Tumor Suppressor, as an upstream driver of cardiomyocyte specification. Transcriptional regulation and trajectory inference from *NF2*-null cells reveal the loss of cardiomyocyte identity and the acquisition of non-mesodermal identity. Sustained elevation of early mesoderm lineage repressor *SOX2* and upregulation of late anti-cardiac regulators *CDX2*, *MSX1* in *NF2* knockout cells reflect a necessary role for *NF2* in removing regulatory roadblocks. Since YAP is a known repressor of mesendoderm genes, we found that NF2 and AMOT cooperatively bind to YAP during mesendoderm formation, thereby preventing YAP activation. Mechanistically, we show that the critical FERM domain-dependent formation of the AMOT-NF2-YAP scaffold complex at the adherens junction is required for mesodermal lineage specification. These results provide mechanistic insight into the essential role of NF2 for cardiomyocyte lineage specification by sequestering the repressive effect of YAP and relieving regulatory roadblocks *en route* to cardiomyocytes.

P16

Investigating transcriptional networks involved in blood lineage commitment

Samiyah Shafiq[1,2], Kiyofumi Hamshima[2], Georges Lacuad[1], Kiran batta[1], Jonathan Yuin-Han Loh[2]

[1]University of Manchester, UK; [2]Institute of Molecular and Cell Biology, Singapore

Direct reprogramming of somatic cells presents great therapeutic potential to generate patient specific blood cells for regenerative medicine. However, this potential is hindered by the stochastic nature and low efficiencies of reprogramming. Here we reprogram mouse embryonic fibroblasts (MEFs) into induced hematopoietic stem and progenitor cells (iHSPCs) to decipher the mechanisms involved during reprogramming and comprehend the internal factors driving cells to reprogram. To this end we use a dox inducible system to reprogram cells by the overexpression of only two transcription factors, TAL1 and LMO2. Reprogrammed cells possess hematopoietic cell surface markers, have colony forming potential and express many HSPC marker genes. iHSPCs generated can differentiate into cells of myeloid, erythroid and megakaryocyte lineages. These results indicate that overexpression of SCL and LMO2 provides a suitable alternative method to reprogram MEFs to iHSPCs, which can be used to further understand the stochastic reprogramming process.

P17

Tumour suppressor Parafibromin/Hyrax governs cell polarity and centrosome assembly in neural stem cells

Qiannan Deng[1], Cheng Wang[1], Chwee Tat Koe[1,#], Jan Peter Heinen[2], Ye Sing Tan[1], Song Li[1], Cayetano Gonzalez[2,3], Wing-Kin Sung[4,5], and Hongyan Wang[1,6,7*]

[1]Neuroscience & Behavioral Disorders Programme, Duke-NUS Medical School, Singapore; [2]Institute for Research in Biomedicine, The Barcelona Institute of Science and Technology, Barcelona, Spain; [3]Institució Catalana de Recerca i Estudis Avançats, ICREA, Barcelona, Spain; [4]Genome Institute of Singapore, Genome, Singapore; [5]Department of Computer Science, National University of Singapore, Singapore; [6]Dept. of Physiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore; [7]NUS Graduate School - Integrative Sciences and Engineering Programme (ISEP), National University of Singapore, Singapore; #Genome Institute of Singapore, Genome, Singapore

Neural stem cells (NSCs) divide asymmetrically to balance their self-renewal and differentiation, an imbalance in which can lead to NSC overgrowth and tumour formation. The functions of Parafibromin, a conserved tumour suppressor, in the nervous system are not established. Here, we demonstrate that *Drosophila* Parafibromin/Hyrax (Hyx) inhibits ectopic NSC formation by governing cell polarity. Hyx is essential for the asymmetric distribution and/or maintenance of polarity proteins. *hyx* depletion results in the symmetric division of NSCs, leading to the formation of supernumerary NSCs in the larval brain. Importantly, we show that human Parafibromin rescues the ectopic NSC phenotype in *Drosophila hyx* mutant brains. We have also discovered that Hyx is required for the proper formation of interphase microtubule-organizing center and mitotic spindles in NSCs. Moreover, Hyx is required for the proper localization of two key centrosomal proteins, Polo and AurA, and the microtubule-binding proteins Msps and D-TACC in dividing NSCs. Furthermore, Hyx directly regulates the *polo* and *aurA* expression *in vitro*. Finally, overexpression of *polo* and *aurA* could significantly suppress ectopic NSC formation and NSC polarity defects caused by *hyx* depletion. Our data support a model in which Hyx promotes the expression of *polo* and *aurA* in NSCs, and in turn, regulates cell polarity and centrosome/microtubule assembly. This new paradigm may be relevant to future studies on Parafibromin/HRPT2-associated cancers.

P18

Investigating the role of histone modifications in regulating replication fork dynamics

Hong Nguyen[1], Nitika Taneja[1*]

[1]*Molecular Genetics, ErasmusMC, The Netherlands*

Chromatin remodelers and histone modifiers are the major determinants of accessibility to nucleosome DNA, allowing the accomplishment of essential biological processes, such as DNA replication. Interestingly, recent studies have shown frequent aberrations in chromatin modifiers in cancer. One such factor is the histone methyltransferase, EHMT2/G9a. This protein is responsible for the methylation of H3K9 at euchromatic loci and thereby, regulates the transcriptional activity of genes. Moreover, it has been shown that EHMT2/G9a is associated with the replication fork. However, the role of EHMT2/G9a at the replication fork is still elusive. Therefore, we have assessed the role of EHMT2/G9a at the replication fork. This work showed that EHMT2 is enriched at the stalled fork, where EHMT2 catalyses mono-di methylation on H3K9, which serves as a platform for H3K9me3. Furthermore, EHMT2 catalytic activity is important for the maintenance of chromatin compaction at the stalled fork. Moreover, we demonstrated that the catalytic activity of EHMT2 is necessary for the recruitment of fork protection proteins at the stalled forks to prevent their degradation from nucleases and maintain genome stability in cells. Taken together, this study uncovered a novel role of EHMT2 in maintenance of proper chromatin environment for safeguarding the stalled replication forks integrity.

P19

NAFLD modelling with hepatocyte-like cells derived from adult liver stem cells via air-liquid interface

Soe Einsi Lynn[1,2], Jian Hui Low[1], Chwee Tat Koe[1], Pierre-Alexis Vincent Goy[1], Lai Ping Yaw[1], Tiffany Oon Teng Woo[1], Agnes Bee Leng Ong[3], Torsten Wuestefeld[3], Mei Chin Lee[1], Huck Hui Ng[1,4,5,6]

[1]*Laboratory of Precision Disease Therapeutics, Genome Institute of Singapore, Singapore;* [2]*Department of Medicine, Yong Loo Lin School of Medicine, National University of Singapore, Singapore;* [3]*Laboratory of In Vivo Genetics & Gene Therapy, Genome Institute of Singapore, Singapore;* [4]*Department of Biochemistry, National University of Singapore, Singapore;* [5]*NUS Graduate School for Integrative Sciences and Engineering, National University of Singapore, Singapore;* [6]*Department of Biological Sciences, National University of Singapore, Singapore*

Non-alcoholic fatty liver disease (NAFLD) is widely cited as the next “silent pandemic” affecting 25% of the worldwide population and 29.3% of Singapore’s population. As NAFLD represents an economic burden, the development of non-invasive diagnosis methods and novel treatments is of priority to manage the disease. Although primary human hepatocytes (PHH) are the gold standard for studying liver diseases, cultured PHH would rapidly dedifferentiate and lose their functionality. To create a sustainable NAFLD target identification and validation system, our laboratory has developed a reproducible method to isolate self-renewable adult liver stem cells (LSCs) from donor tissues. Isolated LSCs can be differentiated into hepatocyte-like cells (HLCs) when cultured via the air-liquid interface (ALI) under defined media culture conditions. Differentiated HLCs were validated to express hepatocyte markers and displayed functionality *in-vitro*. Free fatty acid (FFA) treated HLCs, that exhibit steatosis, are found to be responsive to drug treatments such as DGAT (Diacylglycerol

Acyltransferase) inhibitors. These DGAT inhibitors-treated HLCs successfully demonstrated the reversal of steatosis and were extensively characterized as reliable cell resource for NAFLD modeling.

P20

Identifying molecular signatures in diabetic skin fibroblasts to improve skin health

Hwee Hui Lau[1,2], Shih Ling Kao[3,4], Shawn Hoon[5], Jayantha Gunaratne[6] and Adrian Kee Keong Teo[1,4]

*[1]Stem Cells and Diabetes Laboratory, Institute of Molecular and Cell Biology, A*STAR, Singapore; [2]School of Biological Sciences, Nanyang Technological University, Singapore; [3]Division of Endocrinology, National University Hospital, Singapore; [4]Yong Loo Lin School of Medicine, National University of Singapore, Singapore; [5]Molecular Engineering Lab, A*STAR, Singapore; [6]Mass Spectrometry and Systems Biology Laboratory, Institute of Molecular and Cell Biology, A*STAR, Singapore*

Type 2 Diabetes (T2D) has the highest prevalence among the elderly population (aged 60-69) in Singapore. Among the clinical conditions associated with diabetes, skin complications affect 30-90% of individuals, raising major health concerns. The function of skin deteriorates with age and the presence of diabetes, leading to impaired wound healing. Without appropriate interventions, impaired wound healing could worsen into outcomes such as foot ulceration and amputation. Thus far, the molecular mechanism(s) leading to poor skin health in T2D are not well-understood. Here, we made use of skin fibroblasts derived from 14 healthy and T2D Singaporean Chinese male donors as a model to investigate poor skin health in aged T2D population. Our study revealed that skin fibroblasts derived from T2D donors lose proliferative capacity at a faster rate when compared with that from healthy donors, suggesting premature aging. Genome-wide transcriptomic and proteomic profiling demonstrated that T2D fibroblasts had reduced cellular motility and elevated DNA damage (hallmarks for poor wound healing) when exposed to chronic hyperglycemia conditions. Our transcriptomic and proteomic profiling of healthy versus T2D fibroblasts over the course of *in vitro* aging also identified unique molecular signatures associated with aging and diabetes, offering new insights into the mechanism(s) underlying poor skin health. We are currently identifying and validating target genes/proteins that could possess therapeutic potential for improving skin health in aged T2D patients.

P21

Investigating environmental nanoplastic toxin in promoting the onset or aggravation of neurodegenerative disease

Winanto[1,2], Shi-Yan Ng[1,3], Yi-Cherng Liou[2]

*[1]Institute of Molecular and Cell Biology, A*STAR Research Entities, Singapore; [2]Department of Biological Sciences, National University of Singapore, Singapore; [3] National Neuroscience Institute, Singapore*

Incidence of neurodegeneration has increased over the past decades, but this is not entirely explained by longer lifespans. We noted that this coincides with the increased use of single- use plastics from the 1970s. Various studies have reported increase oxidative stress following accumulation of nanoplastic in the brain tissue. Increase oxidative stress may affect many cellular processes leading to apoptosis and neuroinflammation, a hallmark of neurodegenerative disease. In this study, we investigated if polystyrene nanoplastics (PS-NPs) can result in an onset or exacerbation of neurodegenerative disorders. Our current work reveals that 50nm PS-NPs can be efficiently taken by human neurons and iPSC-derived motor neurons (MNs). PS-NPs exposure reduces survival, neuronal health (neurite length, soma size) and mitochondrial function. Importantly, Pull-down assay revealed that PS-NPs can bind to TDP43 and result in hyperphosphorylation of TDP43, which is a protein implicated in both ALS (motor neurons disease) and frontal temporal dementia (FTD). Finally, we found that concurrent treatment of activated carbon and PS-NPs reduces bioaccumulation of PS-NPs in spinal motor neurons. Therefore, our study demonstrated that environmental nanoplastic pollution can bind to key proteins involved in neurodegeneration. Its exposure has demonstrated to reduce neuronal health and survival, which can be ameliorated by treatment with activated carbon.

P22

hESC-derived motor neurons as a novel in vitro model for EV71 infections

Wai Hon Chooi[1], Cheryl Yi-Pin Lee[1], Winanto Ng[1], Yingying Zeng[1], Pradeep Gautam[1], Jonathan Yuin-Han Loh[1], Sylvie Alonso[2,3], Shi-Yan Ng[1]

*[1]Institute of Molecular and Cell Biology (IMCB), A*STAR, Singapore; [2]Infectious Diseases Translational Research Programme, Department of*

Microbiology and Immunology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore.; [3]Immunology Programme, Life Sciences Institute, National University of Singapore, Singapore

Enterovirus 71 (EV71) causes Hand, Foot, and Mouth Disease and has been clinically associated with neurological complications. The infection of the motor neurons at the neuromuscular junctions has been proposed to be the main route for EV71 to invade the central nervous system. However, there is a lack of relevant human models to elucidate the neuropathologic mechanisms of EV71, as the current models rely on animals or immortalized cell lines. Here, we propose using human ESC-derived motor neurons as an *in vitro* model for EV71 infections. We found that the percentage of EV71-infected cells increased with increasing multiplicities of infection (MOI), where cytopathic effect was observed from 24h post-infection. The EV71 in the infected cells were replicating and infectious, as demonstrated by plaque assays, dsRNA staining, and non-structural viral protein staining. Furthermore, EV71 also infected neural progenitors and interneurons there were present in the culture, at a similar rate to the motor neurons. Thereafter, we surveyed the modulation of host gene expression during EV71 infection using scRNA-Seq. Our preliminary results suggest mitochondrial dysfunctions occur in the infected motor neurons. Finally, we demonstrated that this is a suitable model to validate known and novel host targets by using siRNA and drugs.

P23

Novel molecular mechanisms in reactivation of quiescent neural stem cells

Mahekta Gujar[1], Gao Yang[1], Qiannan Deng[1], Ye Sing Tan.[1] and Hongyan Wang[1]

[1]Neuroscience and Behavioral Disorders Program, Duke-NUS Graduate Medical School Singapore, Singapore, Singapore

The balance between proliferation and quiescence of stem cells is crucial for development and tissue homeostasis. Dysregulation of NSC reactivation is associated with neurodevelopmental disorders. Recently, we have shown that microtubule growth in the cellular protrusion of *Drosophila* quiescent NSCs are predominantly acentrosomal and that microtubules are oriented plus-end-out toward the tip of the primary protrusion. However, the microtubule-organizing centre (MTOC) of qNSCs is still unknown. Here, we show that the Golgi apparatus is enriched in the protrusion initial segment and may act as the MTOC in qNSCs. Mutations in Golgi proteins such as ARF1and Sec71/ARFGEF2 cause malformations of cortical development, a type of neurodevelopmental disorder in humans. However, how Arf1 and its guanine-nucleotide exchange factor ARFGEF2/Sec71 contribute to brain development is unknown. We demonstrate for the first time a novel role of Golgi proteins Arf1 and its GEF Sec71 as regulators of NSC reactivation via regulating acentrosomal microtubule growth. We also show Mini Spindles (Msps)/XMAP215, a key microtubule regulator in quiescent NSCs functions downstream of Arf1 in regulating NSC reactivation. Ultimately, E-cadherin (E-cad) is localized to the NSC-neuropil contact sites, in a Golgi and Msps-dependent manner to promote reactivation of quiescent NSCs.

P24

A feeder free culture system for human epidermal stem cells

Jun-Ichi Sakabe[1, 2, 3], Alvin Chua[2], Bien Keem Tan[2], Keya Roy[1, 2, 3], Gorostidi François[4], Andrea Zaffalon[4], Ariane Rochat[4] and Yann Barrandon[1, 2, 3, 4]

*[1] Lee & Lee Chair of Plastic Surgery and Regenerative Medicine, Duke-NUS Medical School, [2] Department of Plastic, Reconstructive and Aesthetic Surgery, Singapore General Hospital and [3] Laboratory of Stem Cell Dynamics, A*STAR Skin Research Labs (A*SRL), A*STAR, Singapore; [4]Laboratory of Stem Cell Dynamics, School of Life Sciences, Ecole Polytechnique Fédérale Lausanne (EPFL) and Centre Hospitalier Universitaire Vaudois, 1011 Lausanne, Switzerland*

Transplantation of cultured human autologous epidermal stem cells can be life-saving in deep extensive burn wounds. Cell therapy is available to surgical teams worldwide, including the Burn Centre at SGH, a centre of excellence in South East Asia. A critical step is to achieve a reproducible, cost-effective and long-term expansion of the epidermal stem cell population. The gold standard technology that allows for the production of hundreds of grafts from a small skin biopsy depends on a lethally irradiated mouse 3T3-J2 feeder cells but it is considered a xenotransplantation by regulatory affairs. Our project aims at defining a new gold standard for human epidermal stem cell expansion by providing a safe, high quality and reproducible alternative to current technology. We have demonstrated using single cell cloning and imaging, the most stringent assay available, that the addition of a Rho-kinase inhibitor is

very efficient to massively expand human epidermal stem cells in a feeder free system. We have also demonstrated that the cells have a normal karyotype and generate a normal epidermis in a 3D reconstruction model, thus opening the door to a technology transfer from bench to bedside.

P25

Pancreas decellularization and recellularization for diabetes mellitus treatment

Lillian Yuxian Lim[1], Shirley Suet Lee Ding[1], Padmalosini Muthukumaran[2], Swee Hin Teoh[2,3], Yexin Koh[4], Adrian Kee Keong Teo[1,5]

*[1]Stem Cells and Diabetes Laboratory, Institute of Molecular and Cell Biology (IMCB), A*STAR, Singapore; [2]School of Chemical and Biomedical Engineering, Nanyang Technological University, Singapore; [3]Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore; [4]Department of Hepatopancreatobiliary and Transplant Surgery, Singapore General Hospital, Singapore; [5]Department of Biochemistry and Department of Medicine, Yong Loo Lin School of Medicine, National University of Singapore, Singapore*

Diabetes mellitus is a global disease requiring long-term treatment and monitoring. Currently, the only reliable treatment for achieving stable euglycemia in Type I diabetes patients and severely diabetic Type II diabetes patients is beta-cell replacement by pancreas or islet transplantation. However, the shortage of donor pancreases limits the use of this therapy. In recent years, several groups have developed protocols for the differentiation of stem cells to beta- or islet-like cells. However, these protocols cannot generate islets that are functionally comparable to native islets, likely in part due to the lack of environmental cues from the extracellular matrix (ECM) that are present in the developing pancreas. Decellularized pancreases retain ECM present in the native pancreas and may promote functional maturation of stem cell-derived beta cells. In this study, we optimized the decellularization of mouse pancreases using various decellularization agents and demonstrated that decellularization with 0.5% SDS was able to effectively remove DNA content while retaining major ECM components. These scaffolds were biocompatible as demonstrated by their ability to support the attachment and proliferation of beta cells. Moreover, beta cell seeded on these scaffolds retain functionality comparable to cells seeded on cell-culture plates. Future work will include studies directed at using the decellularized scaffolds to promote the functional maturation of stem cell-derived islets.

P26

Computationally defined and in vitro validated putative genomic safe harbour loci for transgene expression in human cells

Matias I. Autio[1, 2], Efthymios Motakis[2], Arnaud Perrin[1, 2], Talal Bin Amin[1], Zenia Tiang[1,2], Dang Vinh Do[1,2], Jiaxu Wang[1], Joanna Tan[1], Wei Xuan Tan[2, 3], Adrian Kee Keong Teo[2, 3] and Roger S.-Y. Foo[1, 2]

*[1]Genome Institute of Singapore, A*STAR, Singapore; [2]Yong Loo Lin School of Medicine, National University of Singapore, Singapore; [3]Institute of Molecular and Cell Biology, A*STAR, Singapore*

Stable expression of transgenes is essential in both therapeutic and research applications. Traditionally, transgene integration is done via viral vectors in a semi-random fashion, this carries the risks of transgene silencing and disruption of endogenous genes. Genomic safe harbour (GSH) loci have been proposed as safe sites in the human genome for directed transgene integration. Although several sites have been characterised for transgene integration in the literature, most of these do not meet criteria set out for a GSH and the limited set that do have not been characterised extensively. Here, we conducted a computational analysis using publicly available data to identify 25 unique putative GSH loci that reside in active chromosomal compartments. We validated stable transgene expression and minimal disruption of the native transcriptome in three GSH sites *in vitro* using H1 and H9 human embryonic stem cells (hESCs). We differentiated the targeted hESC into all three germ lineages and confirmed high transgene expression via immunofluorescence and high content imaging. Furthermore, for easy targeted transgene expression, we have engineered constitutive landing pad expression cassettes into the three validated GSH in hESCs. In the future, the described GSH may enable expression of therapeutic genes in translational gene and cell therapy.

P27

Single-cell transcriptomics reveals maturation of transplanted stem cell-derived retinal pigment epithelial cells towards native state

Bhav Parikh[1], Paul Blakeley[2], Regha Kakkad[1,2], Zengping Liu[1,2,3], Tim Hu[1], Xinyi Su[1,2,3,4]

*[1]Institute of Molecular and Cell Biology (IMCB), Agency for Science, Technology and Research (A*STAR),*

Singapore; [2]Department of Ophthalmology, Yong Loo Lin School of Medicine (YLL SoM), National University of Singapore (NUS), Singapore; [3]Singapore Eye Research Institute (SERI), Singapore; [4]Department of Ophthalmology, National University Hospital (NUH), Singapore

Transplantation of stem cell derived retinal pigment epithelial (RPE) cells can potentially restore vision in patients with age-related macular degeneration (AMD). Several landmark Phase I/II clinical trials have demonstrated safety and tolerability of RPE transplants in AMD patients, albeit with limited efficacy. This stems from inadequate understanding of RPE transplant biology, in terms of survival, maturation, and fate specification of transplanted cells. To address this, we transplanted stem cell derived RPE into the sub-retinal space of rabbit eyes and conducted single-cell RNA sequencing on the explanted RPE, compared to their age-matched *in vitro* counterparts. After transplantation, we observed an unequivocal retention of RPE identity, and a trajectory inferred survival of all *in vitro* RPE populations. Furthermore, there was a unidirectional maturation in all transplanted RPE towards the native adult human RPE state. Through gene regulatory network analysis, we identified tripartite transcription factors (*FOS*, *JUND* and *MAFF*) specifically activated in post-transplanted RPE cells. Their role is postulated to regulate canonical RPE genes, crucial to support host retina function, and pro-survival genes required for adaptation to the host sub-retinal microenvironment. These findings shed insights into the transcriptional landscape of RPE cells after subretinal transplantation, with important implications for cell-based therapy for AMD.

P28

Reduced cranial placodal formation in patients affected with Bosma Arhinia and Microphthalmia syndrome

Vanitha Venkoba Rao, Shifeng Xue

[1]Department of Biological Sciences, National University of Singapore, Singapore

The vertebrate head has transient embryonic cell types that contribute to its craniofacial structure and sensory function. A rare congenital condition called Bosma Arhinia and Micropthalmia syndrome (BAMS), characterized by absence of nose, is caused by missense mutations in an epigenetic repressor called SMCHD1. Developmentally, the nose is contributed by neural crest (NC) and cranial placodal (CP) cells. Hence, we hypothesize that BAMS is resultant of cellular/ molecular defects in NC and CP cells. We recently showed that SMCHD1 mutations resulting in BAMS have impaired NC cell migration. To model sensory defects *in vitro*, we differentiated induced pluripotent stem cells (iPSC) from control and affected individuals into CP cells using a directed differentiation method modified from Tchieu et al., 2017. Real time qPCR indicated reduced expression of placodal genes in BAMS cells. Furthermore, we observed fewer CP cells (marked by SIX1 and EYA1) in BAMS compared to control. We conclude that there is a defect in differentiation to CP in BAMS. Our ongoing work will identify the molecular and cellular processes that lead to the formation of a nose.

P29

Fulvene rescues Spinal Muscular Atrophy induced cardiac arrythmia

Qian Hua Phua[1,2], Jeremy Pang[1], Boon Seng Soh[1,2]

[1]Cardiovascular Disease Modeling and Therapeutics Lab, Institute of Molecular and Cell Biology, Singapore; [2]Department of Biological Sciences, National University of Singapore, Singapore.

Spinal Muscular Atrophy (SMA) is a common autosomal recessive motor neuron disorder which occurs as a result of low survival motor neuron (SMN) genes due to the homozygous loss of function of SMN1 gene. While SMA is mostly understood as a motor neuron disease, cardiovascular system has been increasingly recognized to be involved. Patients with more severe SMA suffered mostly from structural cardiac defects such as septal abnormalities and hypoplastic left heart syndrome while cardiac rhythm abnormalities were diagnosed in patients with milder forms of SMA. With a growing awareness of cardiovascular defects reported, we seek to explore the mechanisms underlying the pathogenesis of SMA utilizing both in vivo and in vitro models. In our study, smaller hearts were observed in SMA mice, potentially due to the decrease in progenitor pool size and an increase in apoptosis. Furthermore, both molecular and functional impairment were found in iPSC-derived cardiomyocytes reprogrammed from type I and type II patients as well as DOX induced SMN knockdown iPSC-derived cardiomyocytes. Upon treatment with Fulvene, a NOX4 inhibitor, Ca2+ kinetics demonstrated significant improvement, suggesting the potential therapeutic usage of Fulvene to treat arrythmia typically diagnosed in patients with less severe SMA.

P30

Uptake of nanoplastics and its effects on the function of human stem-cell derived cardiomyocytes

Shirley Pei Shan Chia[1], Jeremy Kah Sheng Pang[1], Boon-Seng Soh[1,2]

[1]Disease Modeling and Therapeutics Laboratory, ASTAR Institute of Molecular and Cell Biology, Singapore; [2]Department of Biological Sciences, National University of Singapore, Singapore

Plastic has become an ubiquitous environmental pollutant and nanoplastics (NPs) that are within the size range of 1nm to 1000nm could form upon weathering. Considering its sheer size, NPs are speculated to be more hazardous than their larger counterparts. Despite the growing concern, there is still limited understanding on the effects of NPs on human heart. Therefore, we aim to utilise human embryonic stem cells-derived (hESCs) cardiomyocytes (CMs) to investigate the effects related to the uptake and accumulation of NPs in human heart. Firstly, more mature cardiomyocytes were generated to better recapitulate the effects of NPs in adulthood. NPs effects were then elucidated over 3, 5 and 7 days of NPs treatment. The size-dependent uptake and accumulation of NPs was then established in CMs. Generally, oxidative stress was upregulated in cardiomyocytes in a time sensitive manner but was inconclusive. On the other hand, a rise in apoptosis was noted on all timepoints and was significant on day 7. Correspondingly, arrhythmia was also induced by day 7 of NPs treatment. Overall, our findings suggest that an exposure and accumulation of nanoplastics within hESCs CMs could lead to modifications in oxidative stress generation, decreased cell viability and presented an arrhythmic phenotype.

P31

Single-cell reconstruction of regulatory elements in human eye

Pradeep Gautam[1,2,14], Kiyofumi Hamashima[1,14], Ying Chen[1,2,3], Yingying Zeng[1,4], Bar Makovoz[5], Bhav Harshad Parikh[6,7], Hsin Yee Lee[1] , Katherine Anne Lau[1] , Xinyi Su[6,7,8], Raymond C. B. Wong[9,10,11], Woon-Khiong Chan[2,3], Hu Li[12], Timothy A. Blenkinsop[5] & Yuin-Han Loh[1,2,3,13]

*[1]Cell Fate Engineering and Therapeutics Laboratory, A*STAR Institute of Molecular and Cell Biology, Singapore; [2]Department of Biological Sciences, National University of Singapore, Singapore; [3]Integrative Sciences and Engineering Programme (ISEP), NUS Graduate School, National University of Singapore, Singapore; [4]School of Biological Sciences, Nanyang Technological University, Singapore; [5]Icahn School of Medicine at Mount Sinai, USA; [6]Department of Ophthalmology, Yong Loo Lin School of Medicine, National University of Singapore; [7]Translational Retinal Research Laboratory, A*STAR Institute of Molecular and Cell Biology, Singapore; [8]Singapore Eye Research Institute, Singapore; [9]Centre for Eye Research Australia; [10]Ophthalmology, Department of Surgery, University of Melbourne, Australia; [11]Shenzhen Eye Hospital, Shenzhen University School of Medicine, China; [12]Center for Individualized Medicine, Department of Molecular Pharmacology & Experimental Therapeutics, Mayo Clinic, USA; [13]Department of Physiology, Yong Loo Lin School of Medicine, National University of Singapore; [14]These authors contributed equally*

The vertebrate eye has evolved by undergoing significant changes. However, much of the functioning of the eye and its compartments remain conserved across species. Here, the first single-cell atlas of the human and porcine ocular compartments was constructed. High transcriptomic similarity in cell types was observed across species. Inter-species transcriptome expression data in retinal cell populations were studied to understand the core transcription factors (TFs) that identify a cell type. In the non-retinal cell populations, putative adult stem cells present in the iris of both pigs and humans were identified. The regulons of different cell populations, which included TFs and receptor-ligand interactions, were probed. Finally, one core TF such as KLF7, which was conserved across species, was focused on further studies. After perturbing KLF7 gene expression during retinal ganglion cells (RGC) differentiation, current studies concluded that KLF7 plays a vital role in the maturation of RGC cells during differentiation.

P32

Identification of pancreatic endocrine genes during the differentiation of human pluripotent stem cells into beta-like cells

Lay Shuen Tan[1,2,3], Gabriel Jing Xiang Ong[1,4], Euodia Xi Hui Lim[1,2], Adrian Kee Keong Teo[1,2,3,5,*]

[1]Stem Cells and Diabetes Laboratory, Institute of Molecular and Cell Biology, Proteos, Singapore, Singapore; [2]Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore; [3]Precision Medicine Translational Research Programme, Yong Loo Lin

School of Medicine, National University of Singapore, Singapore, Singapore; [4]Life Sciences, College of Humanities and Sciences, National University of Singapore, Singapore, Singapore; [5]Department of Medicine, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

The differentiation of human pluripotent stem cells into pancreatic endocrine progenitor (EP) cells and pancreatic beta-like cells (βLC) in vitro allows for stage-specific transcriptomic profiling to study pancreatic development. While genes mediating pancreatic development and beta cell function are well-described, the gene regulatory networks governing the EP to βLC transition remains poorly understood. Here, we differentiated wild-type H9 human embryonic stem cells and Asian Chinese human induced pluripotent stem cells into βLCs and performed bulk RNA-Sequencing at various developmental stages and elucidated unique gene expression patterns in the EP and βLC stage. We then performed gene ontology biological processes analyses to elucidate the associated pathways involved during the specific stages. For instance, our study revealed 343 and 133 upregulated genes in (1) EP stage only and (2) βLC stage only found to be involved in (1) cellular localisation and transport and (2) response to stimuli. Importantly, we also filtered our gene lists to reveal stage-specific upregulated or downregulated transcription factors. We are currently validating the expression of these transcription factors to establish their role in pancreatic development. Overall, our work serves as a valuable resource for the identification of genes, molecular pathways and transcription factors uniquely regulated during pancreatic differentiation.

P33

Vitronectin as a key molecular determinant in altered adhesion and migration of human umbilical cord-derived MSCs under stress condition

Ankita Sen[1] and Malancha Ta[1]

[1]Department of Biological Sciences, Indian Institute of Science Education and Research, Kolkata, India

Mesenchymal stem cell (MSC)-based therapy gets compromised as adverse microenvironmental conditions at the target site affect migration, engraftment and viability of transplanted MSCs. To improve treatment efficacy, it is important to assess the impact of microenvironment and identify key molecular players affecting these characteristics of MSCs. We observed that under serum starvation stress, human umbilical cord-derived MSCs exhibited increase in cell spread area and adhesion, with reduction in cellular migration. The changes in these parameters were accompanied by formation of large number of super mature focal adhesions (FAs) and notable induction in vitronectin (VTN) expression. NF-κβ was found to be a positive regulator of VTN expression while ERK pathway regulated it negatively. Inhibition of these signalling pathways or VTN knockdown under serum starvation established that VTN mediated the changes in adhesion and migration pattern along with formation of mature FAs. Further analysis revealed that phosphorylation of myosin light chain (MLC) was induced under serum starvation and inhibiting MLC kinase led to reversal in the observed changes in adhesion, migration and FA formation. Overall, it was demonstrated that VTN-mediated induction in MLC phosphorylation led to increase in FA formation and cellular adhesion while compromising migration in WJ-MSCs under serum starvation stress.

P34

Identification of potential maturation genes in cardiovascular progenitor transplanted myocardial-infarcted pig hearts

Lynn Yap[1], Swarnaseetha Adusumalli[1], Min En Cheng[1], Samantha Lim[1], Woan Ting Tay[1], Cheryl Lee[1], Lena Ho[1], Xin Yi Choo[1], Owen Rackham[1], Ye Lei[2], Eric Lim[3], Vincent Ren[1], Yibin Wang[1], Enrico Petretto[1], Karl Tryggvason[1,4]

[1]Cardiovascular and Metabolic Disorders, Duke-NUS Medical School, Singapore; [2]Department of Biomedical Engineering, University of Alabama, Birmingham; [3]National Heart Center, Singapore; [4]Department of Medicine Duke University, Durham, USA

In this study, we report the derivation and applications of hESC-derived cardiovascular progenitors (CVP) using a reproducible laminin-221 differentiation protocol. We permanently ligated the coronary arteries and transplanted CVPs into an immunosuppressed pig model for 12 weeks. The cells remained viable and formed human grafts in the infarcted region as indicated by IVIS optical imaging and histology staining. Heart function was analyzed by magnetic resonance imaging (MRI) and revealed overall improvement in left ventricular ejection fraction by 10 – 15 % and a reduction in infarction size after CVP transplantation as compared to medium control (p-value < 0.05). Temporary episodes of graft-induced VT over 25 days were developed in 4 pigs and 1 pig had persistent VT, while the rest (n = 5) remained in normal

sinus rhythm. All ten pigs survived the experiment without any VT-related death. Subsequently, we performed 10X spatial transcriptomic analysis at 1-, 2- and 12- weeks post-transplantation to demonstrate engraftment and maturity of the graft. We uncovered novel upstream regulators that are highly expressed in mature grafts and these genes will be studied further to validate cardiac maturation.

P35

Pluripotent states regulation by the 3D genomic interactions of transposable element enhancers

Nadia Omega Cipta[1,2], Yingying Zeng[1,3], Zi Hao Zheng[1,4], Teo Jian Zhou[1], Yuin-Han Loh[1,2,3,4]

[1]Laboratory for Epigenetics, Stem Cells & Cell Therapy, Programme in Stem Cell, Regenerative Medicine and Aging, ASTAR Institute of Molecular and Cell Biology, Singapore; [2]Department of Biological Sciences, National University of Singapore, Singapore; [3]School of Biological Sciences, Nanyang Technological University, Singapore; [4]NUS Graduate School for Integrative Sciences and Engineering, National University of Singapore

The genome of the mammalian species is heavily dominated by transposable elements (TEs), and studies in the past few decades have illuminated TE's multifunctional roles as enhancers, promoters, transcription factor (TF) binding sites, alternative splice sites, and barriers to regulatory domains. At the early embryonic stage, the genome is in a hypomethylated state, which results in a more accessible chromatin state. This provides a window of opportunity for the TFs to occupy these sites and exert gene regulation, such as enhancing gene expression. To date, investigating the motif and binding enrichment of a broad repertoire of TFs on TEs have not been performed. This study investigates the TE-derived enhancers from the TF binding and 3D genome interactome perspective using mouse embryonic stem cells (mESC) model. We aim to answer which TE enhancer and TF interactions are regulating the distinct differentiation potency of cells in early embryonic development. We manipulated mESCs' potency through the addition of chemical cocktails, which convert mESC from a ground pluripotent state to a heightened naïve pluripotent (2i) or totipotent-like state (EPSC). Our results suggest that SINE TE-enhancers are contributing to the mESC's potency states through cooperation with TFs and chromatin remodeling factors.

P36

Theraeutic application of pulsed electromagnetic field for cartilage repair

Nurul Dinah KADIR[1,2], Zheng YANG[1,2], Doreen GOH[1,2], Alfredo FRANCO-OBREGÓN[3,4,5], Eng Hin LEE[1,2], James Hoi Po HUI[1,2]

[1]NUS Tissue Engineering Program, Life Sciences Institute, National University of Singapore; [2]Department of Orthopaedic Surgery, National University of Singapore; [3]Department of Surgery, Yong Loo Lin School of Medicine, National University of Singapore; [4]BioIonic Currents Electromagnetic Pulsing Systems Laboratory, BICEPS, National University of Singapore; [5]Institute for Health Innovation & Technology, iHealthtech, National University of Singapore

Mesenchymal stem cells (MSCs) may contribute to cartilage healing by directly differentiating into chondrogenic cells or by secreting a variety of trophic substances that have a paracrine effect on the cells in the vicinity. MSCs respond "environmentally" to biophysical changes and local microenvironmental signals, such as exposure to pulse electromagnetic fields (PEMF). In this study, we demonstrated enhancement of MSC chondrogenesis and paracrine function through PEMF exposure using a custom designed PEMF delivery device that generates a precise magnetic field of low intensity and frequency. The MSC culture platform (scaffold-free pellet culture, hydrogel or fibrous scaffold culture) as well as the pulse intensity, duration, and dosage have a highly dependent relationship with the PEMF inductive effect. Additionally, chondrocyte and MSC migration was improved by PEMF-induced MSC secretome, which could also reduce cellular inflammatory response and death. Our *in vivo* investigation using a rabbit osteochondral injury model demonstrate that brief exposure to low-amplitude PEMF enhances MSC-based cartilage healing capacity. Overall, our findings suggest that PEMF stimulation may have significant clinical and practical implications for enhancing and restoring cartilage regeneration.

P37

Trans-interaction of risk loci 6p24.1 and 10q11.21 is associated with endothelial damage in coronary artery disease

Kai Yi Tay[1,#], Kan Xing Wu[1,#], Florence Wen Jing Chioh[1], Matias Ilmari Autio[2,5,6], Nicole Min Qian Pek[3], Balakrishnan Chakrapani Narmada[2,4], Sock-Hwee Tan[5,6], Adrian Fatt-Hoe Low[5,6], Michelle Mulan Lian[1], Elaine Guo Yan Chew[1], Hwee Hui Lau[7,8], Shih Ling Kao[5,9,10], Adrian Kee Keong Teo[5,7], Jia Nee Foo[1,2], Roger Sik Yin Foo[2,5,6], Chew Kiat Heng[5,11], Mark Yan Yee Chan[5,6], Christine Cheung[1,7,*]

*[1]Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore; [2]Genome Institute of Singapore, Singapore; [3]University of Cincinnati College of Medicine, USA; [4]Experimental Drug Development Centre, A*STAR, Singapore; [5]Yong Loo Lin School of Medicine, National University of Singapore, Singapore; [6]National University Heart Centre, National University Health System, Singapore; [7]Institute of Molecular and Cell Biology (IMCB), A*STAR, Singapore; [8]School of Biological Sciences, Nanyang Technological University, Singapore; [9]Cancer Science Institute of Singapore, National University of Singapore, Singapore; [10]Department of Medicine, National University Hospital and National University Health System, Singapore; [#]Equal contribution; *Corresponding author*

Rs6903956, which lies in a non-coding region on chr6p24.1, has been identified as a genetic risk factor for coronary artery disease (CAD). We aim to interrogate the molecular basis of 6p24.1 containing rs6903956 risk alleles in endothelial disease biology. We generated induced pluripotent stem cells (iPSCs) from CAD patients (rs6903956 risk AA genotype) and normal controls (rs6903956 non-risk GG genotype). CRIPSR-Cas9-based deletions (Δ63-89bp) on 6p24.1 (including rs6903956) were performed to generate isogenic iPSC-derived endothelial cells. Edited CAD endothelial cells exhibited a global transcriptional downregulation of pathways relating to abnormal vascular physiology and activated endothelial processes. A CXC chemokine ligand lying on chr10q11.21, *CXCL12*, was uncovered as a potential effector gene. Underlying this effect was the preferential inter-chromosomal interaction of 6p24.1 risk locus to a weak promoter of *CXCL12*, confirmed by chromatin conformation capture assays on iPSC-derived endothelial cells. Functionally, rs6903956 risk genotypes were associated significantly with elevated levels of circulating damaged endothelial cells (CECs) in CAD patients. CECs isolated from patients with risk genotypes were found to have 10 folds higher *CXCL12* transcript copies than those with non-risk genotype. Our study reveals the trans-acting impact of 6p24.1 with another CAD locus on 10q11.21 and is associated with intensified endothelial injury.

P38

Extracellular RNA biomarkers for the detection of adventitious virus infection of cells

Cheryl Chan[1], Rohan Williams[1,2], Stacy L. Springs[1,3]

[1]Critical Analytics for Manufacturing Personalised-medicine Interdisciplinary Research Group, Singapore-MIT Alliance for Research and Technology Centre, Singapore. [2]Singapore Centre for Environmental Life Sciences Engineering, National University of Singapore, Singapore. [3]Center for Biomedical Innovation, Massachusetts Institute of Technology, Cambridge, USA

Safety testing and risk mitigation processes are key pillars of ensuring safety of cell-based therapeutic products where terminal sterilization of the live product is not feasible. Current *in vitro* tests for live, infectious adventitious viruses in particular are limited by the ability and speed by which the viral contaminant can be cultured, emphasizing the need for faster, sensitive, culture-independent adventitious virus detection methods to expedite in-process and/or release safety testing. Our work harnesses virus RNA signatures within extracellular vesicles secreted from virus-infected cells as potential indicators of infection. In a proof-of-concept study, we have profiled extracellular vesicle-derived viral microRNAs (viral ex-miRNAs) in spent media from a range of virus-infected human T lymphocytes using stem-loop reverse transcription coupled with hydrolysis probe-based quantitative real-time PCR. Exemplified by human T lymphocytes infected with Epstein-Barr and Herpes simplex viruses, we demonstrate detection of the respective viral ex-miRNAs with a lower limit of quantification of 20-30 aM and a time-to-result of less than 4 hours. Ongoing efforts are also focused on exploring the utility of viral ex-miRNA signatures as a fast, non-destructive RNA-based solution for detecting viral reactivation related to cell-based products/treatment.

P39

Machine-learning based detection of adventitious agents in T cell therapy cultures using long read sequencing

James P. B. Strutt[1], Meenubharathi Natarajan[1], Wei Xiang Sin[1], Elizabeth Lee[1], Paul W. Barone[2], Jacqueline M. Wolfrum[2], Rohan B. H. Williams[1,4,5], Scott A. Rice[3,5], Stacy L. Springs[1,2]

[1]Singapore-MIT Alliance for Research and Technology, Singapore; [2]MIT Center for Biomedical Innovation, Massachusetts Institute of Technology, USA; [3]CSIRO Microbiomes for One Systems Health, Agriculture and Food, Australia; [4]Singapore Centre for Environmental Life Sciences Engineering, Life Sciences Institute, National University of Singapore; [5]Singapore Centre for Environmental Life Sciences Engineering, Nanyang Technological University, Singapore

Cell therapy biomanufacturing is a growing industry. Cell therapy products have the potential to provide life changing treatment for patients. Yet challenges remain, these are derived from the short product shelf-life, small lot size and subsequent strict sterility requirements during release testing. Our aim is to meet and solve the challenges of rapid, sensitive and unbiased detection of adventitious agents by combining third generation nanopore amplicon sequencing alongside machine learning. We generated samples incorporating T-cells spiked with bacterial and fungal species. Following sequencing, the sequenced reads are processed, host reads are removed and potential contaminant species identified in an untargeted metagenomics approach. The analysed sequencing data are aggregated to build machine learning models that seek to classify samples and predicted contaminants in order to answer the two following questions (1) is my sample contaminated? (2) is my contaminant genuine? Taken together we are able to prepare a 1 mL spiked sample and detect contaminants at 10 CFU within 24 hours.

P40

Unravelling human endocrine progenitor cells

Alexandra Goncalves[1,2,6], Matthew J Birket[1], Rachel E Jennings[1,3], Ludovic Vallier[4,5], N Ray Dunn[6,7], Neil A Hanley[1,3]

*[1]Faculty of Biology, Medicine & Health, University of Manchester, UK; [2]Institute of Molecular and Cell Biology, A*STAR, Singapore; [3]Endocrinology Department, Manchester University NHS Foundation Trust, UK; [4]Cambridge Stem Cell Institute, University of Cambridge, UK; [5]Berlin Institute of Health-Center for Regenerative Therapies, Berlin Institute of Health, Charité-Universitätsmedizin Berlin, Germany; [6]Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore, [7]Skin Research Institute of Singapore, Agency for Science, Technology and Research (A*STAR), Singapore*

Diabetes is one of the most prevalent diseases of the modern era and the global impact is only expected to increase. With no cure available, patients rely on insulin injection or pharmacological interventions that modulate ion channels within beta cells to promote the secretion of insulin into the bloodstream. Cadaveric islet transplants are the best option for the treatment of autoimmune Type 1 diabetes. This treatment is however severely restricted by the supply of donor islets and the immune response. It is imperative to develop new cell therapies for diabetes. To date, many groups globally have pursued the derivation of insulin-secreting beta cells *in vitro* from the directed differentiation of human pluripotent stem cells (hPSC). These endocrine cells are often not fully mature or sufficiently functional enough to respond physiologically to a major glucose challenge. It is therefore imperative to have a deeper understanding of human pancreas development and the signalling pathways that govern the emergence of the different endocrine subtypes – alpha, beta, delta, epsilon and PP cells that are all derived from a common, ancestral progenitor. The transient expression of the transcription factor NGN3 identifies these endocrine progenitors. scRNAseq from human embryos and *in vitro* pancreatic differentiations have shed light on the pseudo-time of the cell lineage; however, some questions remain unanswered. One major outstanding question is endocrine cell fate primed prior to, during or post-NGN3 expression? Using a human hPSC NGN3 knock-in reporter cell line, it is possible to monitor *in vitro* the development and differentiation from endocrine progenitor to hormone-positive cell types. We propose that decoding the molecular mechanisms and temporal activity during NGN3 expression will provide crucial insights into the acquisition of endocrine cell identity. We further pose the question whether NGN3 and/or post-NGN3 positive cells are able to proliferate and if so, are these divisions symmetric or asymmetric?

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