Research Project in the SMART CAMP IRG
(August 16, 2019)

1. **Critical Quality Attributes of Mesenchymal Stromal Cells for Cell Therapy Applications**
   This project will focus on therapeutic applications of mesenchymal stromal cells (MSCs) and the means for improving therapeutic efficacy through novel biophysical profiling strategies. When MSCs are expanded *ex vivo* for clinical use, MSC progenitors emerge and can exhibit varied biological properties (differentiation, secretome, etc.); this cell population heterogeneity can reduce efficacy for a given therapeutic target outcome, and can be correlated in some cases with biophysical properties at the single-cell level. The project aims to investigate how underlying biochemical changes lead to appreciable biophysical changes to MSCs that may be leveraged for translational efforts.

   MIT Principal Investigator(s):
   Jongyoon HAN (Biological Engineering and Electrical Engineering)
   Krystyn VAN VLIET (Materials Science and Engineering and Biological Engineering)

   Singapore Co-Investigator(s):
   Hanry YU (NUS, Yong Loo Lin School of Medicine, Physiology)

2. **Single Cell Multi-omics**
   The potency and efficacy of manufactured cell products largely depend on cell biological activities, notably the secretion of specific gene products including soluble factors. Most current technologies (e.g., ELISA) measure the population-averaged secretion products of a large population of cells, but for cell therapy manufacturing process development we seek to assess the functional heterogeneity among these cells. Here we will develop new molecular biology techniques and microfluidics platforms to implement functional phenotyping of single cells. We will also conduct multi-omics profiling of single cells that links functional measurement to molecular phenotype (e.g., transcriptome). Successful completion of this project will shed light on the biochemical determinants of cell function, thus enabling better control of cell manufacturing processes for multiple cell types and targeted indications.

   MIT Principal Investigator(s):
   Laurie BOYER (Biological Engineering)

   Singapore Co-Investigator(s):
   Lih Feng CHEOW (NUS, Department of Biomedical Engineering)

3. **Biophysical Critical Quality Attributes of Immune Cells for Cell Therapeutic Applications**
   Immune cells including T cells are candidate therapeutics for which the donor cell source exhibits wide variation in biological and biophysical attributes. When the donor cells are modified as part of the therapeutic cell production, including genetic engineering, this underlying variation can contribute to and compound challenges with therapeutic cell homogeneity and yield of potent cells. This project will focus on identifying properties of T cell therapeutics that may correlate with improved efficacy or safety, and that can be determined in a rapid and label-free manner. Consideration of biophysical traits correlative with desired T cell biological traits (effector vs. memory, presence of appropriate co-stimulation) can provide CQA and increase efficacy, particularly for autologous cell therapies derived from immune cells

   MIT Principal Investigator(s):
   Michael BIRNBAUM (Biological Engineering)

   Singapore Co-Investigator(s):
   Hanry YU (NUS, Yong Loo Lin School of Medicine, Physiology)

4. **Multimodal Biophysical Cytometry and Instrumentation**
   To translate Cellular Critical Quality Attributes into cell manufacturing process analytics with translational impact at sufficient speed and resolution, new optical approaches are required. This project will study of the biophysics of cellular autofluorescence, and the engineering of tools to utilize this feature for cellular cytometry. We envisage this technology to characterize the activity/energetic state of cell therapy products, including cells that either do or do not require surface adherence to maintain viability and proliferation. The project will develop optical instrumentation for monitoring the redox profile of single cells in the adherent or suspended state, improve data analytics for this optical cell characterization modality and study the underlying biophysics.

   MIT Principal Investigator(s):
   George BARBASTATHIS (Mechanical Engineering)
   Peter SO (Mechanical and Biological Engineering)

   Singapore Co-Investigator(s):
   Paul MATSUDAIRA (NUS, Department of Biological Sciences)

5. **Microfluidic High Resolution Cell Sorting**
    Biophysical sorting with clogless filters provides one key approach to isolating cells of potent and predictive efficacy for cell therapy products. This project will advance biophysical analysis and sorting platforms to increase the speed and process flow integration for cases in which cell diameter and/or mechanical deformability are established CQA. Thus the focus will be on developing platform technologies for high-throughput sorting of expanded adult tissue cell and blood cell sub-populations that help define potent cell therapy products.

    MIT Principal Investigator(s):
    Jongyoon HAN (Biological Engineering and Electrical Engineering)

    Singapore Co-Investigator(s):
    Hanwei HOU (NTU, School of Mechanical and Aerospace Engineering)

6. **High Sensitivity Detection of Viral and Bacterial Nucleic Acids**
    In the context of Cell based therapy, there is a need to develop assays to detect bacterial and viral contaminants, or adventitious agents, in cell therapy products. Even at low absolute concentrations, presence of such contaminants signals a risk. DNA-based species identification and detection of extremely low-abundance adventitious agent detection can provide critical insight in cell therapy manufacturing. This project will develop and employ a culture-free / amplification-free DNA-PNA (peptide nucleic acid) hybridization assay.

    MIT Principal Investigator(s):
    Jongyoon HAN (Biological Engineering and Electrical Engineering)

    Singapore Co-Investigator(s):
    Ye AI (SUTD, Engineering Product Development)

7. **CRISPR-based Adventitious Agent Detection for Cell Therapy Applications**
    One of the key challenges in deploying cell therapies lies in the analytical assays needed to confirm that they are safe for administration. This project will develop assays to detect bacterial and viral contaminants in cell therapy products, including new technologies for rapid, sensitive, and specific diagnostics to detect microbial and viral contaminants. We will use synthetic biology technologies to build and optimize detection enabled by gene editing, with the goal of integrating the technologies into state-of-the-art manufacturing workflows for cell therapies.

    MIT Principal Investigator(s):
    Timothy LU (Biological Engineering)

    Singapore Co-Investigator(s):
    Scott RICE (NTU, School of Biological Sciences)

8. **Nanopore Sensors for Fingerprinting Microbial Contaminants in Cell Therapy Products**

One of the key challenges in deploying cell therapies lies in the analytical assays needed to confirm that they are safe for administration. This project will develop assays to detect bacterial and viral contaminants in cell therapy products. Nanopore sensors offer rapid, high-throughput, and label-free methods for characterizing individual biomolecules and biological structures.

MIT Principal Investigator(s):
Patrick DOYLE (Chemical Engineering)

Singapore Co-Investigator(s):
Slaven GARAJ (NUS, Department of Physics)

9. **Adventitious Agent Detection in Cell Therapy Products**

One of the key challenges in deploying cell therapies lies in the analytical assays needed to confirm that they are safe for administration. As part of the development of cell manufacturing for therapeutics, there is an essential need to ensure that the product is free of adventitious agents, especially given that the recipients are typically immune compromised and hence, highly vulnerable to infection. Further, the cell therapy products have limited shelf-lives and the patients are in critical need of rapid treatment. Thus, there is a vital need for methods that can rapidly and reliably assess products for contamination and do so with high sensitivity and speed.

MIT Principal Investigator(s):
Stacy SPRINGS (Centre for Biomedical Innovation)

Singapore Co-Investigator(s):
Scott RICE (NTU, School of Biological Sciences)

10. **Cell Culture Platform and Instrumentation: Instrumented T-flasks**

Process analytic technologies (PAT) for cell therapy manufacturing must be accessible to or integrated with the culture vessels in which the cells are expanded to sufficient cell number and quality. One such vessel is an instrumented flask that serves as a disposable bioreactor during the cell expansion phase. This project develops such a cell culture platform suitable for controlled culture of T-cells and Mesenchymal Stem Cells (MSCs).

MIT Principal Investigator(s):
Rajeev RAM (Electrical Engineering)

Singapore Co-Investigator(s):
Paul MATSUDAIRA (NUS, Department of Biological Sciences)

11. Process Control in Adherent Cell Culture Systems for Cell Therapy Applications

Process analytic technologies (PAT) for cell therapy manufacturing must be accessible to or integrated with the culture vessels in which the cells are expanded to sufficient cell number and quality. This can be especially challenging for adherent cell types such as mesenchymal stromal cells, which require adhesion to culture surfaces (flasks or beads) and subsequent removal from those surfaces for most types of cell therapy product administration. This project will focus on assessing the efficacy and potency of mesenchymal stromal cell (MSC)-based therapies produced in an automated manufacturing platform.

MIT Principal Investigator(s):
Krystyn VAN VLIET (Materials Science and Engineering and Biological Engineering)

Singapore Co-Investigator(s):
Eng Hin LEE (NUS, Yong Loo Lin School of Medicine, Orthopaedic Surgery)

12. Process Control in Suspended Cell Culture Systems for Cell Therapy Applications

Process analytic technologies (PAT) for cell therapy manufacturing must be accessible to or integrated with the culture vessels in which the cells are expanded to sufficient cell number and quality. For cells that thrive in suspension culture and intended for autologous therapies, this approach requires development of real-time and ideally inline PAT for recently identified critical quality attributes (CQA) of the cell products. This project will focus on assessing the efficacy and potency of T-cell / HSPC therapies produced in an automated manufacturing platform.

MIT Principal Investigator(s):
Michael BIRNBAUM (Electrical Engineering)

Singapore Co-Investigator(s):
Dean NIZETIC (NTU, Lee Kong Chian School of Medicine)

13. Computational Imaging and Instrumentation for Integrated Spectroscopy for Metabolites, Cell Viability and Proliferation

Process analytic technologies (PAT) for cell therapy manufacturing must be accessible to or integrated with the culture vessels in which the cells are expanded to sufficient cell number and quality. This project develops spectroscopic sensing modalities – including Raman spectroscopy - that can be integrated into cell culture platforms ranging from instrumented T-flasks to microfluidics bioreactors. Optical design and instrumentation to characterize the metabolites in the cell culture medium and within the cells will be utilized along with computational tools for spectral analysis in these microenvironments.

MIT Principal Investigator(s):
George BARBASTATHIS (Mechanical Engineering)

Singapore Co-Investigator(s):
Paul MATSUDAIRA (NUS, Department of Biological Sciences)

14. **Microcarrier Protocols/Materials for Adherent-Cell Culture Systems in Cell Therapy Applications**

   Process analytic technologies (PAT) for cell therapy manufacturing must be accessible to or integrated with the culture vessels in which the cells are expanded to sufficient cell number and quality. This can be especially challenging for adherent cell types such as mesenchymal stromal cells, for which production scaleup includes cell adhesion to microcarrier beads and subsequent removal from those surfaces for most types of cell therapy product administration. This project will focus on therapeutic applications of MSCs and the means for improving their efficacy through process optimization in an automated cell culture platform.

   MIT Principal Investigator(s):
   Krystyn VAN VLIET (Materials Science and Engineering and Biological Engineering)

   Singapore Co-Investigator(s):
   Sing Yian CHEW (NTU, School of Chemical and Biomedical Engineering)

15. **Data Analytics, Bio-informatics and Machine Learning in Cell Therapy Manufacturing**

   In Cell Therapy manufacturing, critical quality attributes (CQA) of cells that elicit anticipated therapeutic response, and process analytic technologies (PAT) that monitor and maintain those attributes during cell expansion requires expertise in data analytics. Due to the various CQA and PATs involving large quantities of complex data are generated, artificial intelligence-based machine learning/deep learning-based data analytics are used to enable rapid image analysis and interpretation of data.

   MIT Principal Investigator(s):
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