Research Projects in the SMART CAMP IRG

(October 1, 2019)

1. Project Title: Implementing 3D Microcarrier Culture in a Microfluidic Bioreactor

MIT Faculty Advisor: Krystyn VAN VLIET and Rajeev RAM
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Project Description

Mesenchymal Stem Cells (MSCs) are useful as regenerative medicine for cell-based therapy. To meet the needs of producing good quality stem cells in large quantity for such treatment, a bioreactor-based cell manufacturing approach can be applied for large cell quantity production. MSCs being an anchorage-dependent cell type, require some form of substrate for the cells to attach to and proliferate. As a result, microcarriers are developed as an anchoring substrate for MSCs expansion in a bioreactor setup. In the effort to realize cell manufacturing with microcarriers in a bioreactor system, in this project, focus will be given to establishing protocols for implementing 3D microcarrier culture of MSCs inside a microfluidic chip-based bioreactor. The student will learn how to fabricate microcarriers, to perform 3D microcarrier culture with MSCs, and also to operate the microfluidic bioreactor in both fed-batch and perfusion mode. Project experiment will focus on establishing culture protocol including introducing microcarrier beads and cells into the microbioreactor, testing different culture conditions (pH, dissolved oxygen, mode of cell feeding, etc.), and efficient harvesting of cells from the microbioreactor. The student will also have opportunity to learn to perform PCR for gene analysis of MSCs as well as differentiation assay for MSCs.

Goals

1) To learn about cell culture of MSCs on both planar (2D) and microcarrier (3D) platform
2) To successfully fabricate microcarriers with droplet microfluidics technique
3) To establish protocols for 3D microcarrier culture of MSCs in a microbioreactor

Prerequisites/Skills

Cell biology, cell culture, microfluidics

Types of Software Applications

Software to operate the microfluidic bioreactor

Individual or Team Project

Individual

Relevant Papers and or URLs

1) Mozdzierz, N. J. et al. Lab Chip. 2015; 15(14): 2918–2922.

2. **Project Title: Mesenchymal Stem Cell Metabolomics Analysis by using Raman Spectroscopy, UV-VIS Absorbance and Liquid Chromatography**

MIT Faculty Advisor: George BARBASTATHIS and Rajeev RAM
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Project Description

Mesenchymal Stem Cells (MSCs) are cultured and expanded in bioreactors for use in cell therapy. Cells interact with its environment dynamically thus culture media content contains useful information of the growing cells. The main interaction between the cells and media include uptake of nutrients from the media and secretion of various metabolites from the cells into the media. Efficient analysis of media content in which cells grow could allow prediction of potency and quality of the cells, which translates to how efficacious they can be in cell therapy.

The project consists of collecting and analyzing data from different culture conditions using different instrumentation. Correlations between cells conditions and different measurements obtained with Raman spectroscopy, UV-VIS absorbance and High-performance liquid chromatography (HPLC) will be studied.

The three different measurement techniques are sensitive to different physical characteristics of the analytes. Raman spectroscopy detects vibrational oscillation of molecules, UV-VIS spectroscopy senses light absorption while HPLC is sensitive to mass-transfer efficiency.

The challenge of analyzing a complex mixtures such as cell culture media is that the measured signal is a composition of meaningful part and insignificant background. Presence of strong background can hide important information and set the detection limits for each measurement technique. Part of the project is to compare sensitivity and detection limits of different types of measurements.

Goals

1) Establishing sample collection and measurement protocol by defining measurement sample space (e.g. how frequent to collect and measure sample, how to store samples, any difference between fresh samples and frozen samples, etc.)
2) Collection of data using Raman spectrometry, UV-VIS absorption, HPLC instruments
3) Analysis of measurements

Prerequisites/Skills

1) Experience with measurement hardware is a plus
2) Basic knowledge of optics / chemistry
3) Computer programming

Types of Software Applications
Any generic data analysis tool: (Python, R, Julia or Matlab)

Individual or Team Project
A team of two students

Relevant Papers and or URLs
Not identified

3. Project Title: Data Analysis of Optical Diffraction Tomography on Live Cells

MIT Faculty Advisor: George BARBASTATHIS
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Project Description
Cell therapies, where cellular material is injected, grafted or implanted into a patient to treat a range of illnesses and medical conditions, are a vital and integral component of medicine today - promising treatment of tissue degenerative diseases, cancer, and autoimmune disorders. However, significant challenges currently exist to prevent its widespread adoption including problems such as safety, potency, efficacy, and costs.

Of interest in cell therapy are Mesenchymal Stem Cells (MSC), with clinical applications in the treatment of inflammatory and degenerative diseases. MSCs are cultured in bioreactors and grow in an adherent state (attached to the surface of the bioreactor). The shape of the cells is complex and dynamic – they not only migrate/move around but also divide and interact with neighbouring cells within the colony.

Light microscopy is useful as a visual diagnostic tool in cell biology studies to perform morphological analysis, detection, and identification. Various two-dimensional (2-D) information can be obtained to decipher details such as the cell's planar morphology. However, conventional microscopy is not suitable to obtain three-dimensional (3-D) information, necessitating the use of 3-D imaging modalities. Data analysis of these 3-D information will yield useful information such as cell volume, and 3-D morphology.

These morphological parameters can then correlate with the cell function, quality and performance for cell therapy.

Goals
In literature, it was observed that the MSC cell size correlates with the potency and clinical performance in tissue regeneration treatments. However, the cell size is measured when the MSCs were trypsinized – when the MSCs are quasi-spherical after being detached from the bioreactor surface. Currently, we aim to determine the size and morphology of the

MSCs while they are in the adherent state. This requires the measurement and analysis of more complex 3-D parameters such as elongation, thickness and spread area to describe the MSC morphology. A useful parametrization method may be developed for use in correlating the cell morphology and its function, quality and performance for cell therapy.

Prerequisites/Skills
Interest in physics, math, basic programming knowledge

Types of Software Applications
Any generic data analysis tool: (Python, R, Julia or Matlab)

Individual or Team Project
One to two students

Relevant Papers and or URLs
Not identified

4. Project Title: Metabolome-based Adventitious Agent Detection of Cell Therapy

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Project Description
Cell therapy is one of the novel and promising therapeutic methods that is currently underexplored, due to the presence of risks caused by microbial contamination. Cell culturing requires a highly nutrient medium which attracts microorganisms and their spores and hence leads to microbial contamination, comprising the growth and quality of the cells. It is of great importance to identify the types of microorganisms that presented in the cell culture and investigate their effects in the culture. These contaminations can be caused by fungi, bacteria, yeast and virus, and among which, bacterial contaminations are commonly observed and shown to be an interesting target.

Furthermore, due to the urgent need of patients for a rapid treatment and the limited shelf-lives of cell therapy products, a screening method with high sensitivity and speed is needed. Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF MS) is a rapid, sensitive and cost-effective analytical tool for analyzing biological samples with minimal amounts. It has been widely used for microbial fingerprinting or detection of composition difference in microorganisms. By comparing the mass spectra of the microorganisms or their metabolites with the public database, we could be able to identify the species of the microorganisms. When applied in cell therapy products, this technique can assist to identify whether microbial contaminations are present. However, sometimes single analytical method may not reflect the complexity of a biological sample, especially

when no published database is available for the identification. Thus, multivariate analysis tools such as principle component analysis (PCA) are required in this study. Instead of relying on the comparison to a reference compound or on quantifying a particular chemical marker, multivariate analyses usually combine mathematical and statistical techniques to increase the understanding of the data. By combining MALDI-TOF MS with multivariate analyses, we should be able to establish a rapid and sensitive way for microbial screening in cell therapy products.

Goals

1) Learn the basic techniques of handling analytical instruments such as MALDI-TOF MS and HPLC
2) Choose proper metabolite targets for microbial screening
3) Develop and test different microbial metabolite extraction and quenching techniques

Prerequisites/Skills

Bacteria handling, knowledge in analytical chemistry or biochemistry

Types of Software Applications

MATLAB

Individual or Team Project

One to two students

Relevant Papers and or URLs

1) Mahmood A, Ali S. Microbial and viral contamination of animal and stem cell cultures: common contaminants, detection and elimination[J]. J. Stem Cell Res. Ther., 2017, 2: 00078.
2) Giebel, R., et al. "Microbial fingerprinting using matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS): applications and challenges." Advances in applied microbiology. Vol. 71. Academic Press, 2010. 149-184.
3) Smart, Kathleen F., et al. "Analytical platform for metabolome analysis of microbial cells using methyl chloroformate derivatization followed by gas chromatography–mass spectrometry." Nature protocols 5.10 (2010): 1709.

5. Project Tile: Evaluation of CAR T-Cell Products using Flow Cytometry

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Project Description

Chimeric Antigen Receptor (CAR) T-cells utilizes T-cells isolated from the patient and transduced with the CAR transgene, giving the T-cell the ability to recognize and kill its target cancer cell. This revolutionary technology, approved for use in patients in 2017 by the FDA, still faces challenges in the manufacturing and production process to become an affordable and widely available therapy for cancer patients.

At CAMP, we look at biophysical critical quality attributes (CQAs) that informs us on the quality of the produced CAR T-cells, giving better efficacy and potency and ultimately translating to better patient outcomes. In this project, we will look at potency and efficacy assays of different CAR T-cells against its target cancer cells in vitro. Using CQAs as a way to determine good versus bad CAR T-cells products, we will correlate that using our potency assays which includes determining cell death of target cells, cytokine release profiling, status of CAR T-cells pre and post exposure to target cells.

Goals

1) Flow cytometry profiling of various CAR T-cells
2) Flow cytometry assay of target cancer cells
3) Cytokine profiling of CAR T-cells post exposure to target cells

Prerequisites/Skills

1) Basic laboratory skills (Use of pipettes, benchtop centrifuge)
2) Sterile cell culture techniques and hygiene
3) Understanding of Flow Cytometry

Types of Software Applications

1) Microsoft Word, Powerpoint, Excel
2) Graphpad Prism
3) FlowJo Analysis

Individual or Team Project

Individual

Relevant Papers and or URLs

1) Castella, Maria, et al. "Development of a Novel Anti-CD19 Chimeric Antigen Receptor: A Paradigm for an Affordable CAR T Cell Production at Academic Institutions." Molecular Therapy-Methods & Clinical Development 12 (2019): 134-144.
2) Li, Dan, et al. "Genetically engineered T cells for cancer immunotherapy." Signal Transduction and Targeted Therapy 4.1 (2019): 1-17.

6. Project Title: Probing Correlation between Electrical Properties of Mesenchymal Stromal Cells and their Differentiation Potential

MIT Faculty Advisor: Jongyoon HAN
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Project Description

Mesenchymal stromal cells (MSCs) are stem cells that can differentiate into bone, fat or cartilage. Previous work from our lab has demonstrated biophysical heterogeneity in MSC population (suspended cell diameter, cell stiffness in adherent state, nucleus fluctuations in adherent state) that correlates with differentiation outcome. For example, MSCs of diameter 17 to 21 micron (in suspended state) show highest chondrogenic capacity compared to other sized subpopulations. Current project aims to measure electrical properties of suspended MSCs and correlate them with early markers of osteogenic, adipogenic and chondrogenic differentiation. Such correlation of cell biophysical properties with their biochemical attributes has implication in label-free cell selection methods for cell-therapy applications.

Goals

1) Measure cytoplasmic conductivity and membrane capacitance of suspended Mesenchymal stromal cells
2) Label suspended MSCs with fluorescent tagged early differentiation markers (for example, RUNX2-osteogenic, PPARγ-adipogenic, SOX9-chondrogenic)
3) Correlate cell conductivity and capacitance with expression of early differentiation markers

Prerequisites/Skills

1) Cell culture
2) Microfluidics
3) Immunostaining
4) Microscopy
5) MATLAB programming

Types of Software Applications

1) Image J
2) MATLAB

Individual or Team Project

Individual

Relevant Papers and or URLs

1) "Multivariate biophysical markers predictive of mesenchymal stromal cell multipotency" Lee et al. PNAS 2014.
2) "Microfluidic label-free selection of mesenchymal stem cell subpopulation during culture expansion extends the chondrogenic potential in vitro" Lu et al. Lab on a Chip 2018.
3) "Characterizing Deformability and Electrical Impedance of Cancer Cells in a Microfluidic Device" Zhou et al. Analytical Chemistry 2018
4) "Biophysical phenotyping of single cells using a differential multi-constriction microfluidic device with self-aligned 3D electrodes" Yang et al, Biosensors and Bioelectronics 2019.

7. Project Title: Probing Correlation of Morphology and Metabolism of Mesenchymal Stromal Cells with their Differentiation Potential

MIT Faculty Advisor: Krystyn VAN VLIET
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Project Description

Mesenchymal stromal cells (MSCs) are stem cells that can differentiate into bone, fat or cartilage. Previous work from our lab has demonstrated biophysical heterogeneity in MSC population (suspended cell diameter, cell stiffness in adherent state, nucleus fluctuations in adherent state) that correlates with differentiation outcome. For example, MSCs of diameter 17 to 21 micron (in suspended state) show highest chondrogenic capacity compared to other sized subpopulations. Current project aims to measure morphological and metabolic properties of adherent MSCs and correlate them with early markers of osteogenic, adipogenic and chondrogenic differentiation. Such correlation of cell biophysical properties with their biochemical attributes has implication in label-free monitoring of cell culture and label-free selection methods for cell-therapy applications

Goals

1) Culture MSCs from different donors, at different passage numbers, under different culture conditions (proliferation / differentiation)
2) For each condition, label cell membrane, nucleus, mitochondria with fluorescent markers and image ~100 cells per condition at 10X
3) Correlate morphology / metabolism with early differentiation markers (for example, RUNX2-osteogenic, PPARγ-adipogenic, SOX9-chondrogenic)

Prerequisites/Skills

1) Cell culture
2) Immunostaining
3) Fluorescence Microscopy

Types of Software Applications
Image J

Individual or Team Project
Individual

Relevant Papers and or URLs
1) "Multivariate biophysical markers predictive of mesenchymal stromal cell multipotency" Lee et al. PNAS 2014.
2) "Microfluidic label-free selection of mesenchymal stem cell subpopulation during culture expansion extends the chondrogenic potential in vitro" Lu et al. Lab on a Chip 2018.

8. Project Title: Autofluorescence Imaging and Spectroscopy of Live Cells

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Project Description
Cell therapies, where cellular material is injected, grafted or implanted into a patient to treat a range of illnesses and medical conditions, are a vital and integral component of medicine today - promising treatment of tissue degenerative diseases, cancer, and autoimmune disorders. However, significant challenges currently exist to prevent its widespread adoption including problems such as safety, potency, efficacy, and costs.
Light microscopy is useful as a visual diagnostic tool in cell biology studies to perform morphological analysis, detection, and identification. Various two-dimensional information can be obtained to decipher details such as the cell's planar morphology. In addition, cells contain bio-molecules capable of emitting fluorescence, which is known as cell autofluorescence. These cell-endogenous fluorophores are the very same bio-molecules responsible for the host of cellular processes that govern cell functions and metabolic activities. Hence, the autofluorescence signals may be exploited to extract useful information to correlate with the cell function, quality and performance for cell therapy.

NAD and FAD molecules concentrated within the mitochondria inside living cells emit fluorescence, which makes it possible to visualize these organelles through imaging in a non-invasive way without the need for additional staining or dyes. Besides imaging, the autofluorescence signal may be collected through spectroscopy – a technique to decompose the light signal into its separate colour components.

Goals
Currently, we are able to correlate the autofluorescence spectrum obtained through spectroscopy to the metabolic state of the cells. Further measurements and analysis can be done to correlate the cell autofluorescence spectrum to the cell morphology obtained through autofluorescence imaging. More specifically, we aim to determine significant parameters (which may be decomposed autofluorescence components and/or mitochondrial density) to correlate with cell properties which can be used as indicators of the function, quality and performance of the cells for cell therapy applications.

Prerequisites/Skills
Interest in physics, biology, basic programming knowledge (Python/MATLAB)

Types of Software Applications
Not identified

Individual or Team Project
Individual

Relevant Papers and or URLs
1) https://arxiv.org/pdf/1804.05548.pdf
2) https://www.sciencedirect.com/science/article/pii/S0378427411012926