The Chinese University of Hong Kong
Department of Biomedical Engineering

Graduate Seminar – PhD Oral Defence

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Date	***:***	18 August, 2021 (Wednesday)
Time	***:***	10:00 am
Zoom Link	***:***	https://cuhk.zoom.us/j/93178983919?pwd=RUZqTHlEcG9TTUJMODFZNzNBVzZ5dz09
Meeting ID	***:***	931 7898 3919
Password	***:***	838359

Title: Droplet Microfluidics Enabled Cell Culture and Analysis

Characterized by the features of miniaturization, compartmentalization, and parallelization, droplet microfluidics have emerged as a powerful tool for various biomedical applications, especially cell culture and single cell analysis. However, the currently established methodologies based on droplet microfluidics have not reached its full potential. Although water-oil-water double emulsions produced by microfluidics are shown promising for the cultivation of multicellular spheroids, long term cultivation of spheroids and the characterization *in situ* remain the main challenges. Single cell analysis has been leveraged to reveal cell heterogeneities by inspections of nuclear/mitochondrial genomics, epigenomics, transcriptomics, proteomics, and metabolomics. However, the whole genome sequences of most human intestinal microbiota remain unknown due to the lack of reliable methods for single cell genomics. Besides, the methodology for single cell full-length mitochondrial DNA (mtDNA) sequencing remains unexplored although some mutations have been implicated in many pathogenetic phenotypes. As to the specific transcription profiling of heterogenous cells at the level of individual cells, it also suffered from obstacles including the lack of simple approaches for high throughput analysis and the requirements of complex microfluidic chip design and operations.

This thesis is therefore aimed to address the above-mentioned technical challenges. A platform technology, termed Double Emulsion-Pretreated Microwell Culture, was established to allow for effective initiation, sustained maintenance, and culture of the produced spheroids. To allow in situ characterizations of individual spheroids, double emulsions containing spheroids were first localized in microwells on chips followed by spheroids releasing by removing oil layers of double emulsions. Coupled with a built-in analytical station, drug testing of individual spheroids was made possible, which might reveal spheroids heterogeneities and present possibilities as 3D cell culture model for screening drugs. Secondly, as a complement to metagenomics and cultivation, single bacteria genome sequencing was made possible by technical innovations on cell singularization, genome amplification and barcoding strategies, which was validated by high genome coverage of *Escherichia coli* and *Staphylococcus aureus* at the single cell level. Subsequently, we proposed a platform for single cell full-length mtDNA sequencing. Through optimizing polymerase chain reaction (PCR) reagents and lysis buffer, mtDNA amplification from single cells was made feasible. Utilizing barcode templates as primers during PCR, mitochondrial genome was labelled to identify the origin of cells. With the workflow, full-length mtDNA sequences were acquired from single peripheral blood monouclear cells of healthy male and female subjects by the third-generation sequencing. Lastly, we demonstrated a novel thermo-induced dual-core coalescence method for single cell reverse transcription polymerase chain reaction (RT-PCR) in double emulsions, representing a facile and reliable approach for characterizing targeted transcriptome of heterogeneous biological systems. Taken together, the technical advancements established in this thesis are expected to benefit biomedical applications including the high throughput drug screening for anticancer therapies, the establishment of reference genomes for human intestinal microbiota, the illustration of mtDNA mutation profiles from single cells and the identification of genetic mutations or rare cells with specific expression features.

***** ALL ARE WELCOME *****

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