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MEETING REPORT

Microfluidics and its applications in quantitative biology

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INTRODUCTION

Biology research is entering a new era when quantitative measurements are needed to advance our knowledge of the biological systems to the next level where biological behaviors can be understood, predicted and even manipulated. Microfluidics, which manipulate and process small amounts of fluid (10^{-9}–10^{-18} litres) by using channels with dimensions of tens to hundreds of micrometers, provides an exciting opportunity to study cellular behaviors at both single- and multi-cellular levels quantitatively [1]. Microfluidic experiments have the unique capability in providing accurate stimuli to the cells in both space and time with biomimetic physiological conditions to reveal the dynamics of the underlying cellular processes, such as signaling pathway, cell growth and development, and ageing. In addition, microfluidic devices can be parallelized for high throughput, high fidelity experimentation to unravel complex biochemical networks controlling biological behaviors. On the technology side, microfluidics have shown great potential to revolutionize our ability to do genetic analysis at the single cell level, high throughput drug screening, disease diagnostics, and biological and chemical detections.

In October 16–18, 2013, the Center for Quantitative Biology at Peking University hosted an international conference on "Microfluidics and Quantitative Biology" in Peking University. The conference brought many experts in this promising new field to discuss their work on development of state-of-the-art microfluidic techniques and applications of these techniques in solving various biological problems, such as cellular motility, cell cycle, ageing, cellular reprogramming, and cancer. Critical issues such as the potential new directions for microfluidics approaches in quantitative biology and fundamental bottlenecks (challenges) in microfluidic technology are also discussed during the conference. There are twenty invited speakers during the two and a half day meeting with lively discussions during the talks. The following is a brief report on the meeting with highlights arranged in the same order as the conference schedule.

DAY ONE: FROM MAXWELL DEMON TO ORGANS ON A CHIP

The first talk of the conference was given by Bob Austin (Princeton University, Princeton, USA) with a somewhat unconventional title "The demon's ratchet: from dark DNA to dark matter". In his talk, Prof. Austin described the use of various patterned (ordered or semi-ordered) micron-scale structures developed in the Austin lab and their unique capabilities in sorting cells and bio-molecules (e.g., proteins) when these "particles" move — actively for bacterial cells and passively through flow for other bio-molecules — through these patterned micro-structures.

Prof. Austin first described a one dimensional structure made of a linear array of V-shaped wedges (funnels), each about ~20 μm wide and with an opening angle of ~90°. A small gap is left open between these micro-funnels. This linear micro-funnel array is then introduced into a quasi-2D chamber separating it into a left and a right compartment. When inactive particles are introduced into one compartment, they become evenly distributed in the two compartments (the gap between the micro-funnels is wide enough to let the particles pass) by diffusion of the particles, as predicted by the second law of thermo-

dynamics. However, the reverse happens when motile bacterial cells, such as *E. coli* cells were introduced into the chamber. Namely, after equal amounts of cells were introduced into the two equal-sized compartments, the right compartment, on the side of the tip of the "V"-shaped funnel, started to lose cells to the left compartment and ended with much less cells than the left one, contrary to the equilibrium case with the passive particles [2]. Prof. Austin made an analogy of this phenomenon to the famous Maxwell's Demon, a fictitious agent that allows/facilitates violation of the second law of thermodynamics first imagined in a thought experiment by the physicist James Clerk Maxwell more than a century ago. Of course, the second law of thermodynamics is not violated as Maxwell's Demons are not innocent bystanders — they cost energy to observe and interact with the underlying system to break its thermodynamic equilibrium. The question was raised from the audience on "who is Maxwell's Demon?" in the experiments presented by Prof. Austin. Are they the bacterial cells themselves? Can they act alone? Or they need to operate through particular device (making the V-shaped funnel flat abolish the phenomenon)? These questions prompted some lively discussions and the answers (at least to the author of this report) are not completely clear, yet.

Prof. Austin then moved on to discuss a two dimensional bump array made of cylindrical micro-pillars (posts) with radius R. These posts are first arranged in a regular one dimensional lattice in the x-direction with lattice spacing S. Rows of these 1D micro-pillar lattices are then arranged in equal space in the y-direction, each with a small displacement d in the x-direction relative to the row above it. This 2D micro-pillar array is then enclosed in a flow chamber for experiments. In particular, a solution with a mixture of different particles with different sizes were fed from the inlet of the microfluidic chamber ($y = 0$) and pushed through the 2D array along the y-direction. The mixture was collected and analyzed at the outlet end of the chamber. It was found that depending on the geometry, defined by R, S, and d, of the 2D array, the originally well mixed solution can be de-mixed according to the particle sizes. In particular, while the smaller size particles migrate through a zig-zag pattern along the direction of the flow, the larger size particles on average move along a direction with a distinct angle with respect to the flow. Austin then explained that this fascinating and rather unexpected phenomenon actually has an interesting intuitive explanation. Because of the special geometry of the 2D array, there are parallel main flow streams with a particular angle with respect to the flow (y-direction). There are also other streams that can take particles from one main stream to another. As to the smaller particles, because of their smaller size, they can move down along one of the main streams and then jump to another, and this zig-zag movement maintains their downward flow on average. However, for the larger sized particles, when they try to jump to a different stream, they are "bumped" back by the posts due to their large size (hence the name "bump array") [3,4]. As a result, the larger sized particles become "stuck" in a given main stream for a long time, which carries the large particles in a direction with a distinct angle with respect to the flow. As a demonstration of the bump array, this microfluidic de-mixing technique was successfully used to separate red blood cells from white blood cells.

In the final part of his talk, Prof. Austin first made an interesting analogy between the bump arrays that can be made to change the directions of the movement of particles according to their sizes to the well known phenomenon of polarization dependent indices of refraction in optics. The implication of this discovery for nano-biotechnology were also discussed, it is "up to our imagination" according to Austin. In particular, one can design arrays of obstacles as optical elements to: 1) bring objects together and mix them; 2) de-mix them; 3) separate particles that interact from those that do not, which could address a fundamental problem in biology: measuring protein-protein interaction. As a concrete example for using microfluidics techniques in solving important biological problems, Prof. Austin described the challenge in understanding cancer metastasis, in particular, how the cancer cells home into their special sites. He believes that very small nano-vesicles called exosomes may play a role in guiding the cells to the sites, and promoting the initial growth of micro-metastases at the site. He thinks that the real challenge is the construction of bump arrays at the 100 nm scale (not the 100 nm micro level devices already developed in his lab), as exosomes are believed to be very rare. In particular, the Austin lab is making head ways in developing the nano-bump arrays with extremely demanding specs: an array of posts, with 200 nm in diameter, which has 10000 vertical pixels and 50000 horizontal pixels, so a field of 500 megapixels, covering an area of ~50 mm–250 mm. Prof. Bob Austin ended this part of his talk by a plead for help in nano-fabrication in large scale wafer, as there is no 300 mm facility currently available in academic labs.

The 2nd talk of the morning session was given by a PKU researcher, Prof. Chunxiong Luo (School of Physics and Center for Quantitative Biology, PKU, Beijing, China) on "Quantitative studies of dynamic cellular response in microfluidic systems". Prof. Luo first described the general advantages of the microfluidics approach in terms of creating a well-controlled environment for the cells, real time measurement of cellular dynamics, and the high throughput nature of the approach suitable for studying cell-cell variations. He then discussed several areas in biology where his group has

used microfluidics approach to understand the fundamental behaviors (phenotypes) and their underlying mechanisms. Some of the specific areas are: 1) bacterial chemotaxis; 2) cell cycle and size control; 3) antimicrobial resistance.

In studying bacterial chemotaxis, the Luo lab created a microfluidics device where the concentration of the chemical stimuli can be controlled quantitatively in both space and time [5]. The bacterial motility behaviors observed in these different spatio-temporal varying environments informed about the internal signaling pathway dynamics of the cells. The detailed connections between the cellular behaviors and its underlying molecular dynamics as revealed by these well controlled microfluidic experiments are the subject of another talk on Day two of the conference by Yuhai Tu. Prof. Luo then discussed about their recent work in understanding cell cycle synchronization in environments where the nutrient levels were modulated periodically between nutrient rich and poor conditions in a microfluidic chamber. Studies showed that the growth stage (e.g., G1 phase of budding yeast) of the cell division cycle is more sensitive to starvation. Under a nutrient poor condition, the phases of cells can depend more sensitively on the nutrient level. Synchronous yeast cell cycle populations were observed for a range of modulation schemes, with over 80% cells in the population synchronized under optimized scheme. A mathematical model was developed to account for the experimental observations. The work [6] may shed light on the coupling between the cell growth and cell division, and it may also provide a nontoxic and non-invasive way to continuously synchronize cell cycles. As a final example, Prof. Luo described their most recent work on studying drug resistance response of both sensitive and drug-resistance strains at the single cell level by using microfluidics. Cell growth dynamics was monitored in microfluidic chambers, each with different concentrations of antibiotics (ceftriaxone) for both the drug-sensitive and the drug-resistance bacteria strains. From these quantitative measurements, three new discoveries were reported in the talk: 1) The ratio of the antibiotic concentration between the cell death state and the cell elongation state is much larger for the drug-resistance strain than for the drug-sensitive strain; 2) Bacteria with slower growth rate demonstrate higher drug resistance in both the sensitive and the drug-resistance strains; 3) The drug-resistance strain showed some adaptation behaviors, while no adaptation was found in the sensitive strain after changing from low to high antibiotic concentrations. In summary, through these different examples, the microfluidics approach has demonstrated its versatility and applicability in studying biological systems quantitatively.

The 3rd talk of the morning session is given by another PKU researcher, Prof. Yanyi Huang (Biodynamic Optical Imaging Center, and College of Engineering, PKU, Beijing, China), on ”Microfluidics facilitated genome sequencing for limited number of cells”. In this talk, Prof. Huang mainly discussed the work of his lab on the application of microfluidics to genomic analysis. The Huang lab has developed a microfluidics-based single cell RNA-Seq transcriptome analysis technology to perform the library-prep reaction steps at nanoliter range within sealed chambers on-chip, eliminating potential contaminations and complicated manual handling. The design and the operation of the device was described in detail. Each microfluidic device contains two units in parallel. Each unit has five chambers with predetermined volumes. The device is placed under a stereomicroscope with temperature control unit underneath. Cells were sorted manually and all the reactions were controlled by microvalve actuation. The volume of each unit is ~650 nL, representing > 100 fold reduction in volume from the previous method of single cell RNA-Seq.

The single-cell genomic analysis method has the potential of unveiling information from individual cells among heterogeneous cell populations. For example, the microfluidics-based platform provides the possibility to measure the transcriptomes of single embryonic stem cells and mouse embryonic fibroblast cells. The Huang lab can currently detect over 12000 genes expressed within an individual mESC, a record according to Prof. Huang. In summary, the microfluidics-based single-cell RNA-Seq system being developed in the Huang lab holds promise to dissect the complex transcriptome landscape of an individual mammalian cell.

In the afternoon, the four talks cover a diverse set of interesting topics, with a common focus on development of the microfluidics approach/technology for biomedical applications. The first speaker of the afternoon session was Prof. Albert van den Berg (MESA + Institute for Nanotechnology, University of Twente, Netherlands), a pioneer in applying microfluidics to diagnostics and drug development. In his talk entitled “Cell and organs on a chip: new tools for drug development”, Prof. van den Berg first described the rapidly growing interest in the development of new tools that speed up and lower the cost of developing new drugs, where unrealistic in vitro models or hard to control animal models have been used, both having serious drawbacks [7,8]. In his talk, Prof. van den Berg presented different examples of new approaches using microfluidics to circumvent these bottlenecks in diagnostics and drug development. In the first microdevice, the metabolism of the liver is mimicked and drug conversion is carried electrochemically. The conversion products of procainamide are analyzed in mass-spectrometer, and four compounds found give a perfect match with in vivo measurements with patients. In a second example a Blood Brain Barrier (BBB) chip was presented.

The formation of a tight junction with endothelial cells on chip is demonstrated and the effects of fluid flow and the cytokine TNF-α on the Trans Endothelial Electrical Resistance (TEER) is clearly shown. Next, a microfluidic model of a blood vessel with stenosis to study atherosclerosis was presented. In this device, the expression of vWF as a function of flow rate was studied in a microengineered channel cladded with an endothelial cell layer, and the formation of blood clots just after the microchannel occlusion was visualized. Finally, a microdroplet platform for encapsulation of single cells in microdroplets, ordering of these microdroplets and 1:1 fusion of these droplets is demonstrated. All the examples showed convincingly that microfluidics is a very promising and powerful new tool that can be used for high throughput single cell experimentation in drug development and disease diagnostics.

The second talk in the afternoon session was given by Dr. Xingyu Jiang (National Center for NanoScience and Technology, Beijing, China). In his talk "Using microfluidics to understand the mechanical microenvironment of the blood vessel", Dr. Jiang continued the theme of the afternoon session by describing a general micro-fabrication strategy to build tubular structures with multiple types of cells as different layers of the tube walls like in the blood vessel [9,10]. By using a stress-induced rolling membrane (SIRM) technique, tubes with controllable sizes were obtained. By rolling, simple patterns on 2D membranes are transformed into complex patterns in 3D tubes. These tubes could mimic blood vessels in which different types of cells constitute different layers of the tubular wall, and cells on certain layers align either longitudinally or circumferentially around the tube, mimicking their natural arrangement in vivo. Further improvement was made by forming two layers of cells before rolling the membrane. In the 3D tube, two types of cells can directly interact and communicate with each other, mimicking the in vivo conditions of blood vessel. Finally, a microfluidic flow-stretch chip that integrates fluid shear stress (FSS) and cyclic stretch (CS), two major mechanical stimulations in cardiovascular systems, was developed for cultured cells. The model chip can deliver FSS and CS simultaneously or independently to vascular cells to mimic the hemodynamic microenvironment of blood vessels in vivo. The flow-stretch chip serves as a reliable tool for simulating a realistic hemodynamic microenvironment.

The third talk of the afternoon session was given by Prof. Chunyang Xiong (College of Engineering and Academy for Advanced Interdisciplinary Studies, Peking University, Beijing, China) on "Quantitative approaches in cell mechanics and mechanobiology". Prof. Xiong's talk focuses on quantitative measurements of the cells' physical factors/environments such as force, geometry and matrix elasticity, which can play critical roles in the regulation of various biological processes, such as gene expression, adhesion, migration, cell fate, and tissue homeostasis. Prof. Xiong first described the traction force microscopy (TFM), which is a quantitative technique for measuring cellular traction forces. He presented an improved-throughput TFM method using the well developed micro-contact printing technique and chemical modifications of linking microbeads. This technique can be used to quantitatively measure three-dimensional traction forces exerted by cells fully encapsulated in elastic hydrogel matrices. High-resolution, high-speed live cell imaging techniques were used to capture the molecular events in B lymphocytes activation after the recognition of Ags tethered to polyacrylamide gel substrates with different stiffness. Study from the Xiong lab showed that the initiation of B cell activation was extremely sensitive to substrate stiffness and the mechanosensing ability of B cells was dependent on microtubules, and was only mildly linked to the actin cytoskeleton. In the second part of his talk, Prof. Xiong also briefly presented a microfluidic device for single cell mechanical characterization through dielectrophoresis (DEP).

The last talk of the day was given by Prof. Yong Chen (Department of Chemistry, Ecole Normale Suprieure, Paris, France and Centre for Quantitative Biology, Peking University, China). Prof. Chen has been at the forefront of developing innovative microfluidics technologies for biomedical research [11,12]. In his talk entitled "Synthetic cellular microenvironment: artificial extracellular matrix and microfluidics", Prof. Chen described his recent work in developing cellular microenvironment that mimics nature as much as possible. Prof. Chen started his talk with a quote from the great Greek philosopher Aristotle, *Nature does nothing uselessly (Aristotle: I.1253a8*), to motivate his search for bio-mimic cellular microenvironment by working with engineered substrates and microflows.

In his talk, Prof. Chen reminded us that the previous studies on engineered substrates were mostly focused on synthetic polymers such as polydimethylsiloxane (PDMS) which are more easily patterned using the existing technologies, but cannot be suited for the fabrication of tissue constructs. Therefore, there is a pressing need to process nature extracellular matrices which are made of proteins for ideal construction of cellular microenvironment. It was also noted that the commonly used culture platforms are based on either static (dish based) or perfusion (embedded micro channels) culture which is not ideal for dynamic flow regulation. Cells in our body are maintained by diffusion of nutrients or ingredients in the tight space between capillaries. Accordingly, we need to create diffusion

networks for long term cell culture and tissue construct fabrication. The Chen lab has been engaged in developing a novel solution-based approach by patterning natural bio-polymers such as collagen and gelatin to manufacture advanced cell culture support and scaffolds. Two examples of bio-culture platforms were discussed with the emphasis of long term expansion of pluripotent stem cells and functional tissue formation. A diffusion-based cell culture platform, showing the feasibility of open access and high precision flow control, was also proposed. Finally, a new research direction on three-dimensional scaffold fast prototyping as well as related biomedical applications was also discussed.

DAY TWO: APPLICATIONS — FROM CANCER MIGRATION TO AGEING IN YEAST TO BACTERIAL CHEMOTAXIS AND MORE

Quantitatively understanding the functions of biological systems from networks of bio-molecules to cells and organs is a daunting task, but is an extremely important first step for developing new strategies for improving human conditions, such as treating cancer and delaying ageing. Considering the similar scales and fluidic environments, micro-scale engineering technology is providing unprecedented opportunities to address the challenges in exploiting and understanding these complex biological systems. The program of Day two continues the theme of applying/developing microfluidics tools to understand various important biological systems.

In the first talk entitled "Microfluidics platform for quantitative cancer biology" of the morning session, Prof. Jianhua Qin (Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, China) discussed her work on a newly developed microfluidic technology that can be applied to replicate many aspects of the confined tumor environment [13,14]. The new technology enabled the quantitative characterization of molecular and cellular events associated with tumor progression. In particular, the specific epithelial-to-mesenchymal transition of glioma cells promoted by hypoxia microenvironment has been demonstrated. These results highlight the hypoxia-inducible factor (HIFs) as key players in the phenotype transformations of glioma cells and shed light on the molecular mechanisms of Glioblastoma recurrence following angiogenic therapy.

The emerging field of micro-technology has opened up new possibilities for studying cellular motility, in particular chemotaxis, a process where cells sense and move up/down a chemical gradient. Chemotaxis is one of the basic biological phenomena shared by many different types of cells, from bacterial cells to neutrophil to cancer cells. Indeed, cancer cell chemotaxis plays instrumental roles in cancer metastasis, where cancer cells break away from the primary tumors, migrate through the interstitial space, enter/exit vascular vessels and establish secondary tumors at foreign sites. Despite its clinical importance, the roles of cancer cell microenvironment (e.g., 8 chemokine gradients) in facilitating its migration to the metastatic site are largely unknown.

In the second talk of the morning session, Prof. Mingming Wu (Biological and Environmental Engineering Department, Cornell University, Ithaca, USA) described her recent work in understanding cancer cell chemotaxis in well defined chemokine gradients, interstitial fluid flows and biometrix stiffness [15–17]. The Wu lab developed and used 3D microfluidic model to re-create physiologically realistic 3D microenvironment for cells. Advanced imaging systems were used to follow dynamics of individual cancer cells within a lymphoidal like environment. Using a malignant breast cancer cell line (MDA-MB-231) as a model system, the Wu lab found that cancer cell migration is tightly controlled by the chemokine gradients, the compliance of the 3D biomatrix, and the interstitial fluid flows.

The third talk of the morning session was given by Prof. Luke P. Lee (Departments of Bioengineering, Electrical Engineering & Computer Science Biophysics Program, and California Institute of Quantitative Biosciences, UC Berkeley, USA), one of the pioneers of microfluidics technology for bioengineering [18,19]. In his talk entitled "Biophotonic gene circuits and cellular BASICs", Prof. Lee described his work on the precise control of gene regulation essential for both fundamental cancer biology and translational medicine. In the first part of his talk, he presented an impressive work on the remote optical control of the NF-κB pathway in a living cell.

The Lee lab achieved the first optical gene regulation of NF-κB by the selective liberations of IκB siRNA and p65 siRNA from optical nanoantennas with plasmon resonance at λ = 785 nm and λ = 660 nm respectively. The efficacy of the bimodal control was demonstrated through measurements of the levels of expression of IP-10 and RANTES activated by nuclear p65. In the second part of his talk, Prof. Lee discussed several enabling micro-technologies including: 1) cellular BASICs (Biologic Application Specific Integrated Circuits) for quantitative single cell analysis, aging, dynamic cell culture array; 2) Electrophysiologically Activated Cell Sorting (ePACS) for label-free stem cell cytometry; 3) patient-specific iPSCs-based Integrative Microphysiological Analysis Platforms (iMAPs) for drug screening and safety; and 4) Integrated Molecular Diagnostic Systems (iMDx) for global healthcare.

The last talk in the morning session was on ageing given by Prof. Hao Li (Department of Biochemistry and

Biophysics, UCSF, San Francisco, USA and Center for Quantitative Biology and School of Physics, PKU, Beijing, China). Despite the enormous progress made in the yeast aging field, the molecular causes of aging and death remain elusive. A major limitation to yeast aging research has been the inability to track mother cells and monitor molecular markers in the process of ageing. Prof. Li and his collaborators recently developed a microfluidic system capable of retaining mother cells in microfluidic chambers while removing daughter cells automatically [20,21]. Coupled with time-lapse microscopy, this system allows for direct visualization of various cellular and molecular events in single cells throughout their lifespan. Using this new technology, the Li lab have revealed the basic molecular/cellular features of ageing, identified a molecular marker predictive of lifespan, and discovered different forms of cell death with distinct molecular characteristics. Through the combination of single cell and genomic analysis aided by microfluidics, a comprehensive description and a mechanistic understanding of replicative aging at the molecular level is now within reach.

The afternoon session started with a talk by Prof. Luhua Lai (Center for Quantitative Biology and College of Chemistry and Molecular Engineering, Peking University, Beijing, China) on "Discovery of novel chemoeffectors using microfluidic devices and computational screen". Prof. Lai reported a recent study on the molecular mechanism of *E. coli* chemotaxis by screening novel chemoeffectors for its Tar receptor using microfluidic devices and computational screen. First, molecular docking was used to screen for molecules that may bind to the Tar receptor. Molecules from the computational screen were tested for their effects on *E. coli* cell movement by using a specially designed microfluidic device and their binding abilities to the periplasma domain of Tar were measured using isothermal titration calorimetry (ITC). Two antagonists for Tar receptor were found for the first time by this combined screening method. The different responses between attractants and antagonists can be explained by the different conformational changes of the Tar receptor after binding to these molecules. Based on the analysis, the Lai lab (rationally) designed a few Tar mutants that can positively response to other types of amino acids, to which the wild type Tar does not respond to. This study provides an excellent example of using multi-disciplinary approaches to understand biology at molecular level [22].

The second talk of the afternoon session was given by Prof. William Ryu (Department of Physics and the Donnelly Centre, University of Toronto, Toronto, Canada) on "A study of *C. elegans* behavior and memory using microdroplet arrays". *C. elegans* with its tractable genetics, simple nervous system of only 302 neurons, is an ideal model organism for the study of behavior. This simple nematode is capable of a number of complex behaviors, including navigation using chemical and thermal cues, nociception to harsh stimulus, and learning to associate sensory signals with the presence of food. For example, worms cultivated at a specific temperature T_c with food will form an associative memory of this temperature, and if placed on a thermal gradient, the worm will perform thermotaxis to this thermal memory. The memory itself is plastic and the worm will re-encode a new thermal associative memory if placed at a new T_c with food. How does the worm encode this analog value of temperature and associate it with the presence of food? What are the dynamics of this memory? What happens when the worm is starved? Does it negatively associate temperature with this condition? To study these questions the Ryu lab developed a simple microdroplet array assay system that allows them to measure the behavior of single worms swimming in ~4 μL droplets over periods of 4 hours [23,24]. When placed on a linear thermal gradient, the worms perform thermotaxis in these droplets, which can be monitored with standard machine vision techniques. The worms' thermotaxis towards their thermal preference reveals the effects of their thermal memory. By measuring this preference dynamically over a time scale of hours, it was shown that the worm has both a positive and a negative associative memory and that these memories have two different time scales of decay. The Ryu lab also shows that the insulin pathway is involved in forming negative-associative memories by studying the behavior of the insulin mutants ins-1 which has lost the ability to form negative associative memories and age-1, which shows a faster acquisition of negative associative memories.

The theme on cell migration continues with the next talk by Prof. Francis Lin (Department of Physics and Astronomy, University of Manitoba, Winnipeg, Canada) on "Immune cell migration in complex guiding environments: an example of coordinated guidance", in which Prof. Lin described his work on T cell migration in lymph node relevant chemokine fields. In particular, the Lin lab used microfluidic devices to examine T cell migration in different co-existing CCR7 ligand fields mimicking the physiological conditions in lymph node sub-regions. They also used quantitative modeling to predict the experimental observations and to provide insights into the underlying mechanism. Their study suggested a coordinated guiding mechanism by the dual CCR7 ligand fields for T cell trafficking in lymph node with particular implication for the T cell exit mechanism from lymph nodes [25].

The subject of the next talk goes back to cancer cell migration/invasion (metastasis) given by Prof. Liyu Liu (Institute of Physics, Chinese Academy of Sciences,

Beijing, China) with the title of "Quantifying cancer cell collective behaviors in microfluidic governed landscapes". The metastatic cancer cells invade the tissues by damaging the extracellular matrix (ECM). No single cell could finish this tough job and has the power of killing patients. Prof. Liu described his work on understanding the collective effects that ultimately bring mass destruction to the ECM structures by constructing microfluidic models to study cell invasion systematically and quantitatively. Prof. Liu reported that the cell invasion is sensitive to chemo-attractant gradient and applies distant forces to the ECM. Also, his results revealed that the metastatic breast cancer cells MDA-MB-231 have cooperative behaviors that enhance their invasive efficiency. To further explore cell behaviors with different population and encountered ECM resistance, the Liu lab is building micro tracks for cell invasion with patterned protein repellent coating (PRC).

The last talk of the day was given by the author of this meeting report, Prof. Yuhai Tu (IBM T. J. Watson Research Center Yorktown Heights, USA and Center for Quantitative Biology and School of Physics, Peking University, Beijing, China). Bacterial chemotaxis behaviors have been studied for over a century by using some ingenious classical behavior assays, such as the capillary assay and the swarm plate assay. Despite the success of these qualitative (or semi-quantitative) experiments, more quantitative and controlled methods are needed to fully understand the underlying mechanism for the chemotaxis behaviors. In his talk entitled "Quantitative understanding of bacterial chemotaxis from microfluidic experiments", Prof. Tu discussed some of the recent developments on combining microfluidic experiments and quantitative modeling approaches to understand important chemotaxis behaviors, such as log-sensing [15] and frequency-dependent responses [5]. Together with intracellular measurements of the signaling pathway, these microfluidic experiments allowed us to develop a comprehensive multi-scale model [26,27] (based on the intracellular signaling pathway dynamics) that can be used to explain and predict bacterial chemotaxis behaviors in any spatio-temporal varying environment.

DAY THREE: DROP-BASED MICROFLUIDICS AND BEYOND

The third and the last day of the conference started with a talk by Prof. David Weitz (School of Engineering and Applied Sciences and Department of Physics, Harvard University, Cambridge, USA) on the fascinating droplet based microfluidics technology and its many potential applications. Encapsulation of single cells in micro-sized drops controlled with microfluidic devices enables a wide range of biological functions and properties to be investigated with single cell resolution over large populations of cells (e.g., see Refs. [28,29] for some recent work). In his talk entitled "Drop-based microfluidics: biology one picoliter at a time", Prof. Weitz described the microfluidic toolkit required for this promising new technology and explored the potential of the several possible applications. According to Prof. Weitz, the main advantages of the drop-based microfluidics technology includes: 1) extremely small volume, which means very small total reagent needed and/or many assays can be done with the same amount of reagent; 2) improved signal-to-noise ratio as decreased volume means decreased noise; 3) greatly increasing sensitivity; 4) being highly controllable; 5) solvent never touching walls; 6) reagents never touching the wall. The challenge is to develop surfactant to protect the droplets. These drops can be used as micro-reactors to carry out different biochemical reactions with high precision control. A series of potential applications were discussed during the talk including high throughput screening, directed evolution, sequencing, cell sorting, single cell analysis, etc.

The second talk continues along the theme of drop-based microfluidics given by Prof. Wenbin Du (The Institute of Microbiology, Chinese Academy of Sciences, Beijing, China). In his talk entitled "SlipChip and droplet array for quantitative separation and analysis of bacterial cells", Prof. Du described SlipChip, a technique developed for easier and parallel assays in droplets without use of pumps or valves [30]. In the first part of his talk, Prof. Du described their work in the development of a simple platform to implant microfluidic droplets on disposable plastic petri dish, generating a spiral array to realize large scale storage and analysis of bacterial single cells with random accessibility. The growth of bacteria can be monitored automatically. Droplet can be extracted individually by capillary for off-dish studies. The Du lab applied this droplet array system to determine the minimal inhibitory concentration (MIC) of bacteria, and cultivation of environmental microorganisms. In the second part of his talk, Prof. Du described a simple SlipChip device for studying chemotaxis. Finally, a multiplex dielectrophoresis-PCR SlipChip for rapid detection of bloodstream pathogens was described. Dielectrophoresis system for broad range isolation was validated by capture of the top 10 pathogens responsible for 80% septic patients. Captured microbial cells are then transferred and mixed with preload reagents to perform multiplex PCR for identification of pathogens species.

The last two talks of the conference were given by PKU researchers from the organizing institute — The Center for Quantitative Biology. In his talk entitled "Using bacterial virulence protein to modulate frequency dependent osmo-response in the budding yeast", Prof. Ping Wei

told us that bacterial pathogens have evolved specific effector proteins that, by interfacing with host kinase signaling pathways, provide a mechanism to evade immune responses during infection [31]. Although these effectors contribute to pathogen virulence, they might also serve as valuable synthetic biology reagents for engineering cellular behavior. The Wei lab exploits one such effector protein, the Shigella flexneri OspF protein, to rewire kinase-mediated responses systematically in yeast. Bacterial effector proteins can be directed to inhibit specific mitogen-activated protein kinase (MAPK) pathways selectively in yeast by artificially targeting them to pathway-specific complexes. Moreover, by introducing periodic osmo-stimulations in a microfluidic chamber, Prof. Wei showed that unique properties of the effector can generate new pathway behavior: OspF, which irreversibly inactivates MAPKs, was used to construct a synthetic feedback circuit that shows novel frequency dependent input filtering. These studies demonstrate how pathogens could provide a rich tool kit of parts to engineer cells for therapeutic or biotechnological applications. As a next step, Prof. Wei would like to use microfluidic devices to provide controlled time-varying signals to the systems to test these results.

The last talk of the conference was given by the director of the host institute of the conference, Prof. Chao Tang. In his talk entitled "Using microfluidics to study cellular decision-making", Prof. Tang described his work in understanding the dynamics of the cell cycle in yeast. Through a combination of theoretical and experimental work, some of them done with microfluidic devices, the Tang lab has made some breakthrough discoveries on the molecular mechanisms of cell division in yeast.

CONCLUSION

After a very intensive two and a half day of talks and discussions, everyone (I know I am) is in awe of the immense power and tremendous potential microfluidics holds for biological research and medical applications. It is the belief of many attendees of the conference that the convergence of science, engineering, and medicine embodied in microfluidics has the potential to transform life sciences, and enable us to find better solutions for preventive personalized medicine and low-cost healthcare systems. The challenge is up to us to make these promises reality.

Finally, the author of this meeting report would like to thank the staff from Center for Quantitative Biology, especially Ms. Siyuan Gong, for their hard work in organizing the conference, without which the conference and certainly this meeting report would not have been possible.

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