

Sector of

Microtechnologies



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Microfluidic systems can overcome many of the limitations of existing techniques for single-cell analysis and manipulation, drug screening and cell-based directed evolution experiments. Compartmentalization of single-cells and into pico- or nano-liter volume droplets allows millions of individual cells to be analyzed and sorted at ultra-high-throughput rates. Cells stay alive for extended periods of time in droplets, and secreted molecules from single compartmentalized cells rapidly achieve detectable concentrations due to the small volume of the droplets. Alternatively, encapsulated cells can be lysed and intracellular biomolecules assayed. This enables the genetic make-up of cells to be analyzed as well as the biochemistry, since the released DNA or RNA can be amplified in the droplets. Thus, analysis is highly flexible, and not just limited to the detection of cell-surface markers. In addition to single-cell screening applications we are also involved in two other areas of research namely directed evolution of *in silico* engineered enzymes and development of new drug delivery systems based on degradable biopolymers.

The main research areas of our laboratory include:

1. High-throughput screening of single-cells
2. Single-cell transcriptomics
3. Organs-on-a-chip
4. Directed evolution
5. Development of drug delivery systems

1. High-throughput single-cell screening

Compartmentalization of single cells in droplets allows the analysis of proteins released from or secreted by cells, thereby overcoming one of the major limitations of traditional flow cytometry and fluorescence-activated cell sorting (FACS). In collaboration with Dr. John Heyman (Harvard University) and Prof. Andrew Griffiths (Paris ESPCI) we have developed a binding assay to detect antibodies secreted from single cells compartmentalized in 50 pL droplets (Figure 1). Secreted antibody is detected after only 15 minutes by co-compartmentalizing beads coated with anti-mouse IgG antibodies and a fluorescently-labeled probe: when the antibody captured on the bead binds to the probe the fluorescence becomes localized on the beads, generating a clearly distinguishable fluorescence signal allowing droplet sorting at ~ 200 s⁻¹ rate and cell enrichment. The microfluidic system described is easily adapted to screen other intracellular, cell-surface or secreted proteins and to screen for catalytic or regulatory activities.

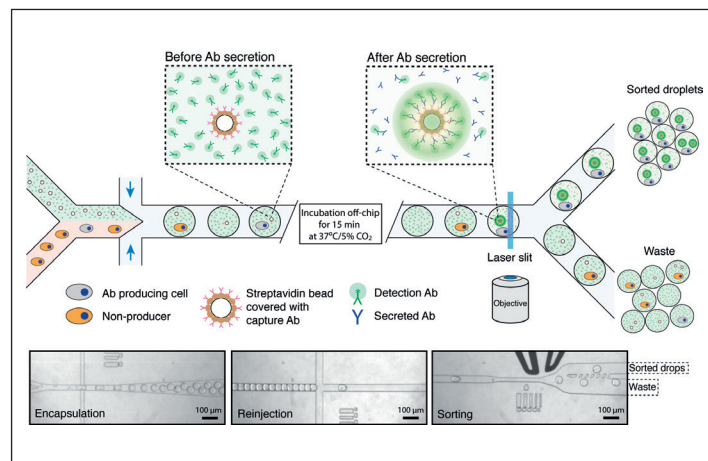


Figure 1. Principle of droplet microfluidics platform for single-cell screening. A cell suspension is introduced into a microfluidic device together with a bead suspension, containing fluorescently-labeled goat detection antibodies (green) and orange fluorescent beads coated with goat anti-mouse-Fc capture antibodies (red). Droplets are created at the flow-focusing junction with fluorinated oil containing fluorosurfactant and then collected off-chip at 4 °C. After incubation for 15 min at 37 °C and 5% CO₂, those beads that are co-encapsulated with an antibody-producing cell become highly fluorescent, due to the capture of secreted antibodies on the bead by the anti-mouse Fc antibody and binding of the green-fluorescent detection antibodies to the captured antibodies in a sandwich assay. The emulsion is then introduced into a second microfluidic device and droplets containing green fluorescent beads are sorted using a fluorescence-activated droplet sorter. Hence, droplets containing no bead, no cell, a cell which does not secrete antibody, or an antibody producing cell but no bead are discarded (no green fluorescent bead is present), whereas droplets containing an antibody-producing cell and a bead (which becomes fluorescent) are collected. The three micrographs show i) co-encapsulation of cells with beads, ii) droplet reinjection after incubation off-chip and iii) droplet sorting. (Adapted from *Nature Protocols* 2013 <http://www.nature.com/nprot/journal/v8/n5/full/nprot.2013.046.html>)

2. Single-cell transcriptomics

In collaboration with Dr. Allon Klein (Harvard Medical School) we developed a novel technique named for parallel barcoding of thousands of individual-cells in a single tube. The principle of this technique relies on encapsulation of individual cells into microfluidic droplets together with beads carrying ssDNA barcoded primers and RT/lysis reagents. The mRNA released from the lysed cells remains entrapped inside the droplet and is tagged with cellular and molecular barcodes during RT reaction. Using such approach simultaneous barcoding of thousands of cells becomes possible. We exemplified the use of the technique to barcode and sequence over

6000 cells (manuscript submitted to Cell), but the technology is highly flexible and can be readily adapted to other applications requiring barcoding of RNA/DNA molecules, such as Chip-Seq, RNA- and DNA-Seq or Hi-C, for example.

3. Organs on a Chip

Platelet transfusions are critically important in biomedicine and patient care. Their demand reach total of >2.17 million apheresis-equivalent units a year in the United States only and are derived entirely from human donors despite clinically significant immunogenicity, associated risk of sepsis, and inventory shortages due to high demand and 5-day shelf life. To take advantage of known physiological drivers of thrombopoiesis in collaboration with Dr. Johnathan Thon and Joseph E. Italiano Jr. (Brigham and Women's Hospital, USA) we have developed a microfluidic human platelet bioreactor that recapitulates the main functions of bone marrow such as stiffness, extracellular matrix composition, micro-channel size, hemodynamic vascular shear, and endothelial cell contacts, and supports high-resolution live-cell microscopy and quantification of platelet production. Physiological shear rates improved proplatelet initiation and release from 10% to 90%, reproduced ex vivo bone marrow proplatelet production, and generated functional platelets. Modelling human bone marrow composition and hemodynamics in vitro obviates risks associated with platelet procurement, storage and may fulfil growing transfusion needs.

4. Directed evolution

In this project we are seeking to combine the latest advances of droplet-based microfluidics technology to explore the directed evolution approach for the optimization of computationally designed enzymes (Figure 3). In collaboration with Prof. Donald Hilvert group (ETH Zurich) we are investigating the mechanisms and strategies for optimizing artificially created enzymes. By applying droplet-based microfluidics technology, in which biochemical reactions are performed inside micrometer size vessels, we are developing a powerful tool enabling large number of libraries (>106) to be screened at ultra-high-throughput rates and using conditions that are incompatible with in vivo systems. Our experimental approach could lead to a deeper understanding of the enzymes catalysis and should afford valuable insights into the evolvability of promiscuous protein functions and scaffolds.

5. Development of drug delivery systems

We are applying droplet microfluidics devices for production of highly monodisperse droplets, particles and vesicles for allow encapsulation of therapeutic molecules, pharmaceuticals and drugs. Additional advantage of using microfluidics is that inner and outer parts of the particles can be tuned by control-

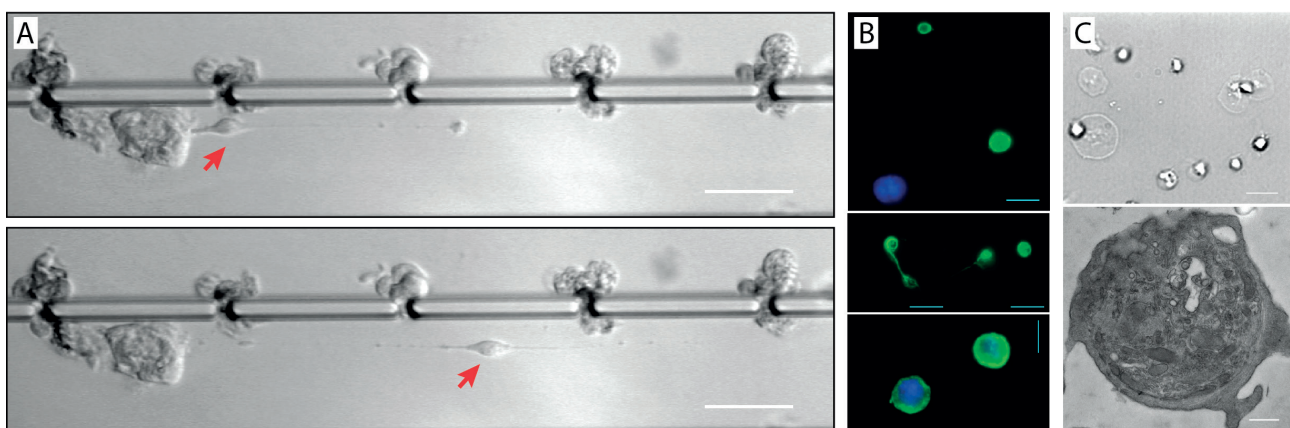


Figure 2. Platelet production using microfluidic bioreactor. *A*) Megakaryocyte cells trapped at the 2 μm gaps between the posts are releasing proplatelets (red arrow) under physiological shear stress (600 mPa) conditions. *B*) Bioreactor derived proplatelets are morphologically similar to human blood platelets, and display comparable microtubule

expression (green). *C*) Bioreactor derived human platelets form filipodia/lamellipodia on activation and spread on glass surface. They are also ultrastructurally similar to human blood platelets and contain a cortical microtubule coil, open canalicular system, dense tubular system, mitochondria, and characteristic secretory granules.

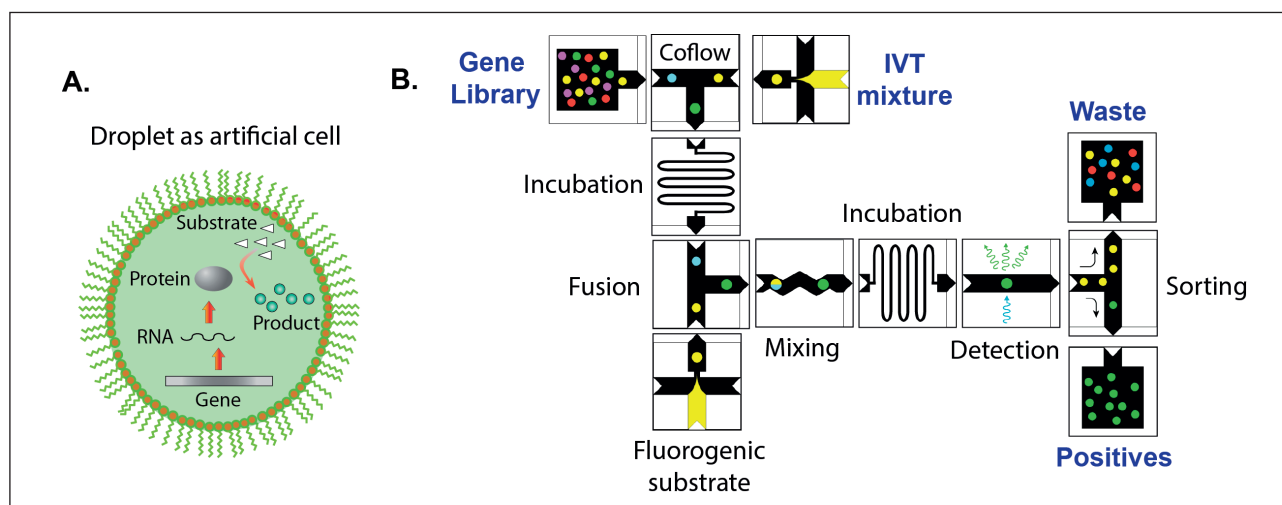


Figure 3. Concept of directed evolution approach in droplet microfluidics. *A) Droplets containing single-genes with all ingredients necessary for in vitro expression will serve as artificial cells that can be selected for a desirable phenotype under conditions that are not feasible in living systems. B) Schematics of the integrated droplet-based microfluidics platform for directed evolution.*

ling the flow parameters, thus allowing precise control over the size and thickness of the shell. We are producing particles with both, solid biodegradable polymer composed of PLGA (lactic-co-glycolic acid) and semi-solid shell composed of alginate.

Collaboration

Prof. David Weitz, Harvard University, USA
 Prof. Andrew Griffiths, Paris-ESPCI, France
 Prof. Donald Hilvert, ETH Zurich, Swiss
 Prof. Andrew deMello, ETH Zurich, Swiss
 Prof. Martin Melis, EPFL Lausanne, Swiss
 Dr. Thon Jonathan, Brigham and Women's Hospital, USA
 Dr. Helder Santos, Helsinki University, Finland

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Publications 2013-2014

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Patent Application

Mazutis L. Microfluidic system and method for production of biopolymer-based droplets and particles. PCT/LT2014/000013. 2014/12/03