Single Cell Encapsulation Using Pinched Flow Droplet Microfluidics

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Abstract—**Recent advances in droplet microfluidics have shown potential for high throughput single cell assays through individual cell encapsulations in water-in-oil emulsions. This compartmentalization allows ones to carry out various enzymatic assays, which quantify intra and extra-cellular proteins. Ordering cells in a micro-channel for droplet encapsulations of individual cells is essential, but is usually limited by the preparation of high-density cell solutions without cell sedimentation and aggregation in a long channel (a few cm). Here, a short micro-channel (a few μm) with pinched structure is manipulated to separate cell aggregation into individual cells and later ordered for single cell encapsulation and assaying in the droplets. The dynamic enzymatic assay of measuring the activity of tyrosine kinases at the individual cell level is performed and the result shows advantage over bulk measurements.**

Current cell based assays follow an ensemble approach to determine the activity thereby masking the differences in expression among individual cells[1,2], which could be misleading due to the averaging of the signals from a subpopulation. Flow cytometers on the other hand do not allow one to perform kinetic studies on individual cell. Recent advances in droplet based microfluidics have shown varies applications including high throughput single cell assays by encapsulating individual cells inside the droplets. Cells randomly distributed in a suspension follow a Poisson's encapsulation statistics. High efficient single cell encapsulation have been demonstrated by using inertial migration in a straight[3] and a spiral channel[4-6]. However, these approaches usually require long channel length (few cm) while facing challenges such as cell sedimentation leading to lower encapsulation rates and might suffer blockage when cells aggregate in the long channels. Here, an approach for ordering cells in a short microfluidic channel (few mm) using inertial focusing through pinched structures that comprise contracting and expanding chambers is introduced. The cell aggregates are separated into individual cells during its transit through the pinching regions of the channel and later ordered for single cell encapsulation and assaying in the droplets.

We described an approach to allow an ordered distribution of cells in a short microchannel (few mm) using inertial focusing through pinched microchannel structures. These structures comprised contracting (focusing width-30μm, and pinching width 12μm) and expanding (width-90μm) chambers (Figure). Cells travelling through these chambers/orifices were focused along the center of the channel following a uniform spacing while moving in groups. Cell clumps are broken down into individual cells due to the shear forces induced on them during their transit through the pinching regions of the microchannel. The cells order along the short straight channel before entering a laminar stream of assay reagents, which were encapsulated along with the cells downstream in the continuous oil phase droplet-forming region.

With the advantages of this device, we demonstrated live cell signal transduction assay measuring the kinetic activity of tyrosine kinase using a commercially available fluorescent peptide reporter Sox substrate, in an antibody-free format. Human lung cancer cells (PC9) were encapsulated in droplets separately along with a specific tyrosine kinase substrate, with and without a specific modulator (Epidermal growth factor, EGF). The binding of epidermal growth factor (EGF) to its receptor, EGFR, at the cell surface, triggered receptor dimerization allowing tyrosine in the cytoplasmic portion of the receptor monomer to be trans-phosphorylated by its partner receptor thus aiding in signal propagation[26]. EGF added into the droplet triggered EGFR signaling resulting in an increase in fluorescence with time. With efficient individual cell isolations, phosphorylation of the Sox substrate as a result of receptor activation, gives us an understanding on the rate of intracellular downstream peptide phosphorylation triggered by surface biomolecular binding in the individual cells. Although the low receptor expression in PC9 cells might result in lesser activity of peptide phosphorylation thus relating to a gradual increase in fluorescence intensity over time, it is difficult to observe these changes from bulk measurements using standard microplate readers while it is much easier to distinguish this at the single cell level.

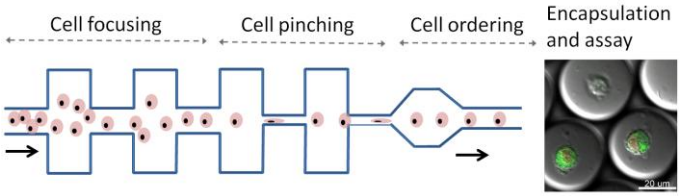

Figure: The uniform cell distribution in physiological solutions is approached through a micro-channel with pinched structure for single cell encapsulations in the individual droplets for performing the kinetic enzymatic activity measurements.

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