Fluorometric Granzyme B Profiling in Single Cells

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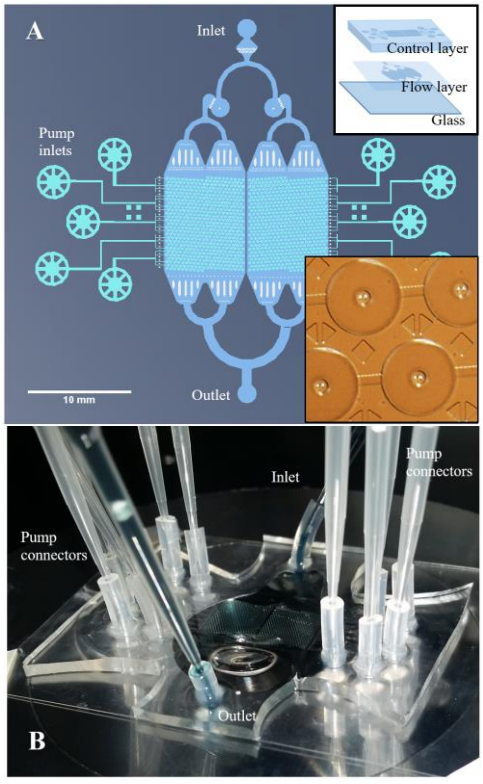
This paper reports on the development of a microfluidic device that has the capability to trap and compartmentalize single cells with the use of PDMS hydrodynamic traps and pneumatic membrane valves. The isolated cells were then studied and profiled for its Granzyme B activity via the conduct of a fluorometric activity assay. Model cells were initially used to test the ability of the device to detect and measure Granzyme B expression, and then later on planned to be applied to peripheral blood mononuclear cell (PBMC) and real blood samples.

To fabricate the microfluidic device, consisting of a flow layer and control layer, photolithography & soft lithography techniques were employed. The flow layer consists of hydrodynamic traps, while the control layer is composed of membrane valves that are actuated to open and close the microchambers. Jurkat cell, Natural Killer cell, THP-1 cell were used as test samples. Single cell Granzyme B activity was detected and measured fluorometrically using a commercial assay kit (PromoKine PK-C57-K168) with a peptide substrate containing Granzyme B recognition sequence (Ac-IEPD-AFC) and AFC (7-Amino-4-trifluoromethylcoumarin) label. Different cell samples showed different Granzyme B activity. Applying the protocol to PBMC and real blood samples would enable the acquisition of baseline to differentiate healthy patients from unhealthy ones.



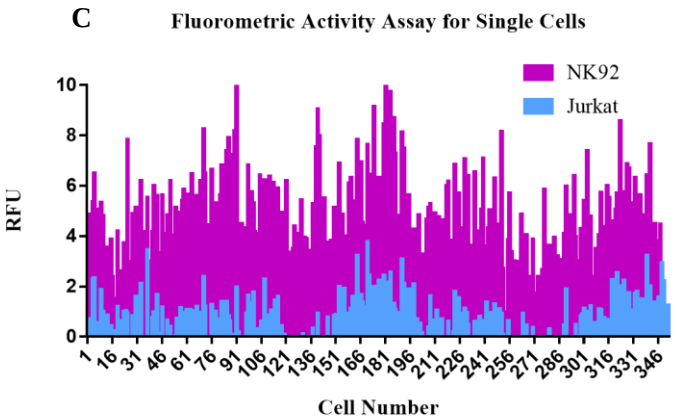


Figure (A). 2D illustration of the entire design; (B) actual photo of the chip (C) Comparison of the fluorescence intensities of NK92 and Jurkat single cells corresponding to its Granzyme B activity..