INDVIDUAL CELL SEQUENTIAL GLYCAN PROFILING ON THE MICROFLUIDIC LAB-IN-A-TRENCH PLATFORM

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State-of-the-art technologies in glycoanalysis include mass spectrometry, protein microarray formats, techniques in cytometry and more recently, glyco-quantitative polymerase chain reaction (Glyco-qPCR). Most of these techniques are restricted to performing functional glycoprofiling at large cell populations while others are unable to handle multiple lectin-probes and are thus limited in terms of exhaustive glycoprofiling of individual live cells. Here, a novel approach of single live cell glycoprofiling leveraged by the microfluidic "Lab-in-a-Trench" (LiaT) platform [1] for performing efficient capture, retention and shear-free reagent exposure for the analysis of the Human Burkitt's lymphoma cells is demonstrated. The most intriguing part of this study is the demonstration of consecutive profiling of glycans on a single cell by sequential elution of the previous lectin using their specific free sugars GlcNAc (N-acetyl-D-glucosamine), GalNAc (N-acetylgalac-tosamine), galactose and mannose.

In this abstract a novel microfluidic Lab-in-a-Trench (LiaT) platform (Fig.1) [1] which facilitates analysis to be performed with low sample volumes, using fewer cells (down to single cell level) and potentially involving multiple probes, in addition to a novel method of profiling carbohydrates is presented. In this sequential method, a given lectin probe bound to its respective carbohydrate on a cell surface is eluted with free sugar followed by the probing of a different cell glycan. The use of LiaT platform also allows for the manipulation of cellular physiological conditions by regulating the fluid properties in the microchannels. Thus, by using the LiaT platform in conjunction with sequential elution of labeled lectin probes from cell surfaces, we have developed an efficient method for the low-volume, shear-free, rapid, sensitive and sequential glycan analysis at single live cell level.

In addition, to profile glycans consecutively, the open-access image analysis tool ImageJ to map glycan density on the surface along with multiple glycan distribution was employed. A high heterogeneity was observed in glycan distribution of any two given cells of the same population. Moreover, under appropriate conditions, quantitative analysis of lectin-carbohydrate interaction in terms of their association and dissociation with the cell surface glycans was also demonstrated for LCA lectin binding to core mannose at single cell and population level.

Firstly, efficient sequential sugar profiles (Fig. 1a) using two labeled lectins were achieved, and then further increased to four lectins. Higher amounts of LCA bound to cells indicating a denser mannose distribution at the surface when compared to ECL, which has an affinity for galactose residues (Fig. 1b). This is in agreement with general glycan structures because galactose is known to be either protected or partially exposed in live healthy cells which results in poor ECL binding and hence lower equilibrium concentrations and reduced signals [2]. Through the use of Image-J software, a qualitative glycan map by simple co-localization of individual lectins in this instance, LCA, ECL, ConA, WGA and an overlap of all (Fig. 1c-d) was created.

Further, an analysis of how low-shear conditions minimize the impact of the specificity of lectin binding events on the cell-surface based compact glycans was performed. Significantly ($p < 0.05$) higher amounts of NPL bound to the cell surface indicating elevated exposed mannobiose as a result of apoptosis, whereas most of the core mannose structures may still have been intact during apoptosis, leading to a strong binding of ConA on the surface (Fig. 2). The obtained results were then analyzed to understand glycan heterogeneity at the single cell level. A significant ($p < 0.05$) difference was obtained at inter-cellular ($n = 12$) level in the glycan-probing behaviors of lectins on individual cells as demonstrated by their LCA lectin profile (Fig. 3**).**

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References

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[2]. R. Gorovosky, K.M. Hoffmeister, H. Falet, "Novel clearance mechanisms of platelets", *Current Opinion in Hematology* **17(6)**, pp 585-589, 2010.

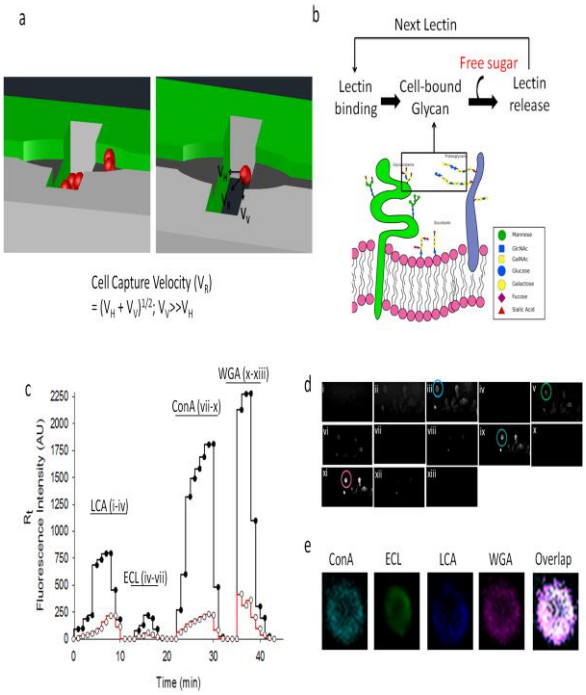

Fig. 1. *Lab-in-a-Trench platform and sequential lectin release for exhaustive glycoprofiling of a live, single human B-lymphoma cell.* ***(a)*** *An illustration of the LiaT platform.* ***(b)*** *Illustration of glycan complexity on the cell surface that also include capping with inner molecules, such as sialic acid.* ***(c)*** *Fluorescence intensities (Rt) depicted in 60-s time steps for glycans profiled with four lectins (LCA, ECL, ConA and WGA). Sequential probing indicates sugars present on cell surface were in the order GlcNAc (WGA) > mannose (ConA) > mannose (LCA) > galactose (ECL).* ***(d)*** *Time-lapse images of lectin binding event on the cell surface. The image panels are mentioned in '**c**' corresponding to their respective lectins.* ***(e)*** *Colour profiles of individual lectins in order to elucidate their distribution on the cell surface.*

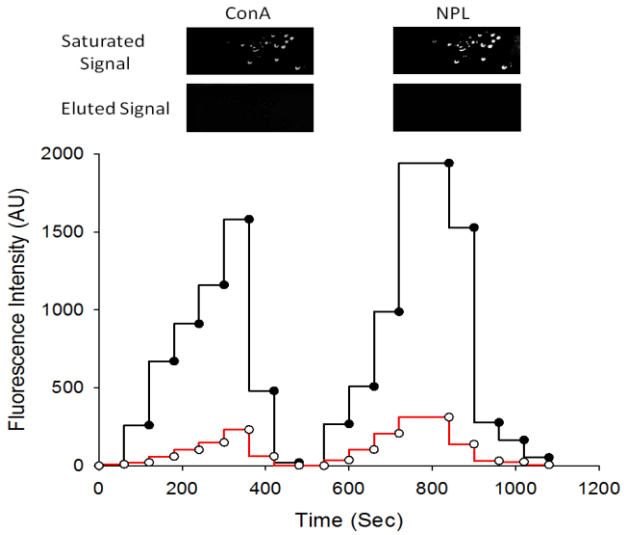

Fig. 2. *Specificity analysis of lectins toward cell-bound sugars. Mannose sugar was targeted using ConA and NPL lectins. The intensity plots indicate complete removal of both sugars and that binding of ConA does not hinder with the availability of sugar for NPL suggesting effective elution and highly specific binding.*

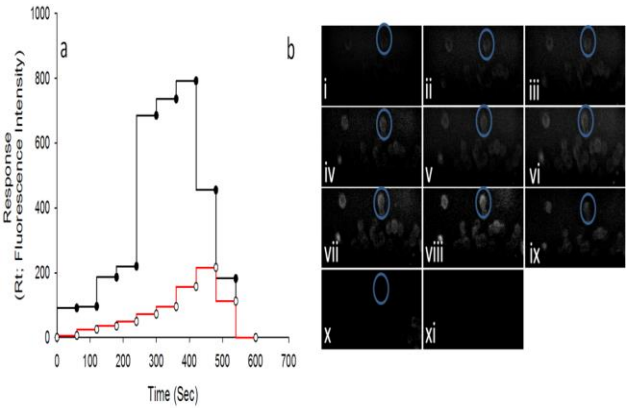

Fig. 3. *Heterogeneity analysis at cell population and single cell level as demonstrated with LCA lectin probing profiles.* ***(a)*** *The intensity distribution pattern of the population (black line) follows binding to the cellular exo-mannose in patches and in single cell (red line; encircled in blue in* ***(b)****) follow a constant binding behavior.* ***(b)*** *The binding (i-viii) and dissociation (ix-xi) events of the studied single cell (blue circles) as a function of appearance and disappearance of fluorescence. A constant increase in the fluorescence was obtained up to image number 'viii' followed by quick dissociation.*