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MICROFLUIDIC LAB-IN-A-TRENCH PLATFORM:
CELL CAPTURE, MANIPULATION AND APPLICATION IN GLYCOBIOLOGY

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Glycans are highly complex carbohydrate molecules and, unlike proteins, they are not encoded by the genome. Instead, they are processed and incorporated at specific locations on proteins and lipids in various combinations at post-translational stages by tightly regulated, enzyme-mediated pathways. Their ability to form complexes within themselves and with other biomolecules, such as proteins, makes them a core component of cellular physiology. Examples of glycan-mediated signaling include embryonic development, cell differentiation and growth, cell-cell recognition, contact inhibition and cell signaling. Glycans therefore constitute an advanced class of information molecules and the full potential of glycan bioprofiling has yet to be realized.

Lectins are a family of proteins that are capable of recognizing and binding reversibly to specific glycan structures. However, the binding affinity tends to much lower than in antibody-based assays. State-of-the-art glycobiology techniques face a series of issues; some are restricted to performing functional glycoprofiling at large cell populations. Others are unable to handle multiple lectin-probes and are thus limited in terms of exhaustive glycoprofiling of individual live cells. It is also well known that flow-induced washing drastically impairs the glycoprofiling of low-affinity lectins (Fig. 3; Left Panel).

We are utilizing here the microfluidic Lab-in-a-Trench (LiaT) platform [1] to tackle these major shortcomings. In brief, the LiaT platform is characterized by a deep indentation in a continuous channel that enables the capture and retention of cells purely through gravity (Fig. 1). The cells are introduced in the system with a gravity pump under laminar conditions controlled by the channel geometry at a velocity below 30 µm s^{-1}. The laminar flow conditions ensure stagnation within the trench, which thus permanently retains cells. In addition, trench depths as small as 200 µm allow continuous, merely diffusion-driven loading of a sequence of reagents and nutrients to enable efficient on-chip cell culturing, cell handling, and multi-reagent handling (Fig. 3). Miniaturization also allows a significant reduction of the number of cells and precious reagent volumes required per test, thus giving opportunity to high-level multiplexing of experiments.

As a pilot study, we performed a 2-reagent-based labeling of T-lymphocyte cells captured. The cells were first labeled by AnnexinV-PE [binds to phosphatidylserine] followed by exposure to a second reagent, i.e. lectin GSLII-DyLight488 [binds to GlcNAc (N-acetylglucosamine)] (Fig. 2). Our apoptosis analysis investigates the simultaneous binding of an early marker AnnexinV and lectin on the apoptosing cells (Fig. 4). On the LiaT platform, we now for the first time demonstrate that lectins, which are usually low-affinity binders and have a tendency to wash off from the cell surface, were labeling the cells significantly better than AnnexinV (Figs. 3 & 4).

Future work will address screening of single and sequential lectin-binding events on a single cell basis to develop a lectin screening database in apoptotic, necrotic, healthy and cancer cells in addition to complex glycoprofiling at single cell level, studying combinatorial effect of hypoxia and anti-cancer drugs on cells and their glycan distribution.
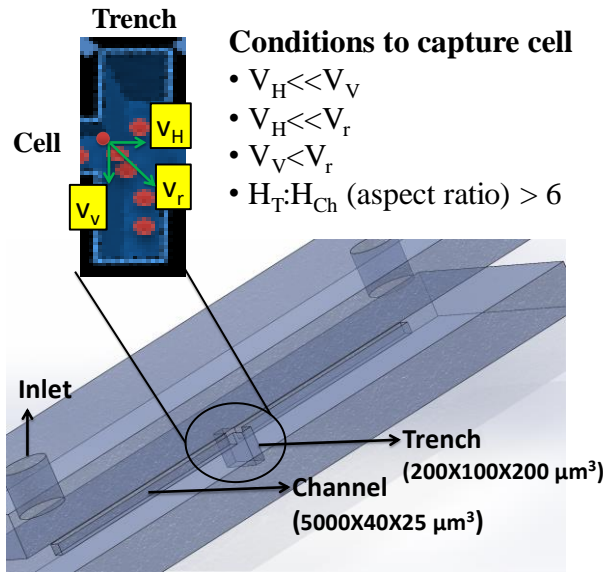
Fig1. *Typical LiaT is a two component system. Upper component possess an inlet and an outlet while lower component has channel and trench. The cell capture is purely gravity-based following the Camp-Hazen model of settling in a basin.*
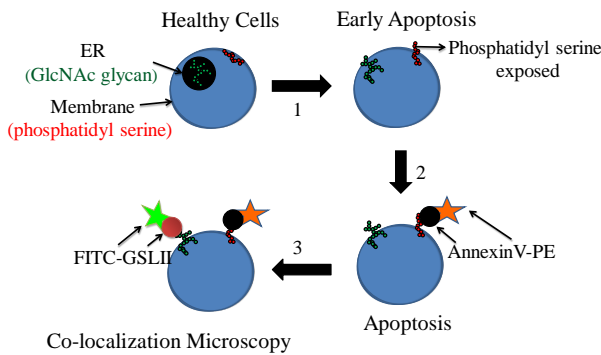

Fig2. *Glycoprofiling procedure. A healthy cell possesses phosphatidyl serine on its inner membrane and GlcNAC in the endoplasmic reticulum (ER). Early apoptosis releases phosphatidylserine on cell surface along with esternalization of GlcNAc at late apoptosis. (1) is IgM-mediated apoptosis induction; (2) Annexin-PE-based phosphatidyl labeling; and (3) GSLII-based GlcNAc labeling followed by co-localization microscopy in order to analyze the extent of labeling.*
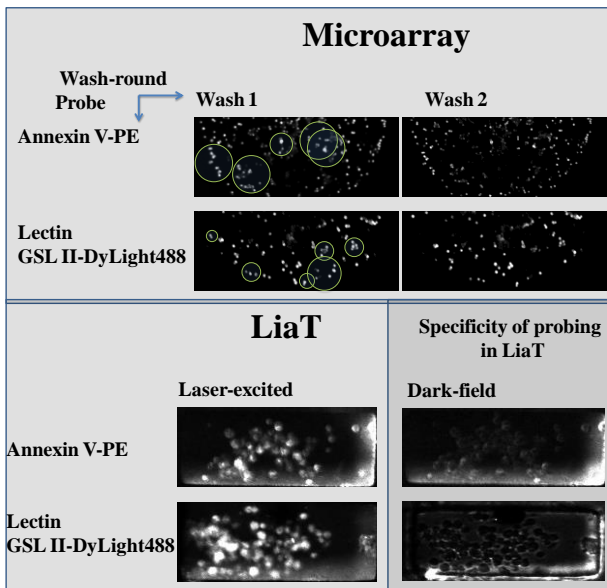
Fig3. *Apoptosis analysis by employing conventional microarray and LiaT platform. An early marker (phosphatidylserine) and GlcNAc sugar analysis of Ramos cells was performed by probing it with AnnexinV-PE dye conjugates and GSLII lectin-DyLight488 conjugate, respectively. The effect of washing on the probing efficiency of Annexin and lectin is also demonstrated for microarray platform (left panel). Green circles represent reduced fluorescence in corresponding spots in 'wash 2' panel. In addition, probing of the same cell population with LiaT platform (central panel) along with specificity of the probing in LiaT platform (right panel) is shown.*
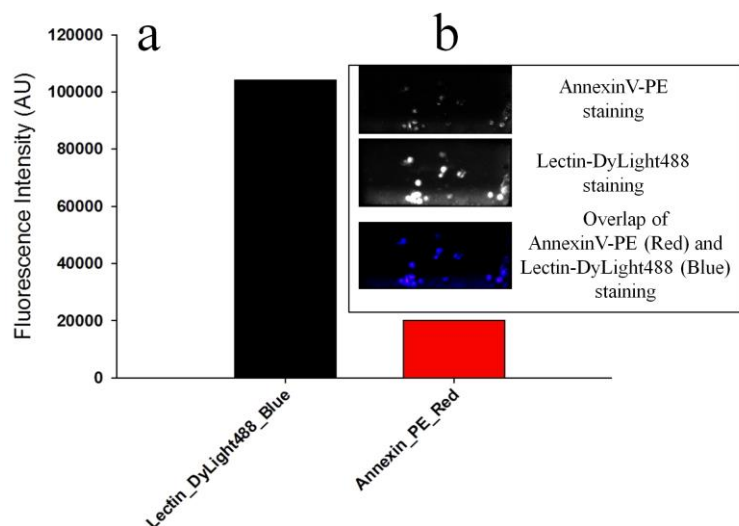

Fig4. *Comparison of AnnexinV binding with that of lectin binding on the apoptosing cells. The results suggest significantly higher lectin binding relative to AnnexinV binding. This implies to that either apoptosing Ramos cells (a derivative of lymphocytes) have higher amount of exposed GlcNAc in comparison to phosphatidylserine it might be associated to the wash-free detection method as washing removes a significant part of cell-bound lectins due to their low affinities.*

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