Sequential glycoprofiling of single cells: A novel approach using Lab in a Trench.

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Using Lab in a Trench technologies, we have developed a novel method to glycoprofile single cells by sequential probing with fluorescently labelled lectins. Current microscopic methods to glycoprofile cells are limited by the inability to probe the glycan structures with more than one lectin, as steric hindrance can interfere with lectin binding. Flow cytometry is similiarly limited, and methods such as NMR and Mass Spectrometry do not have the possibility to probe at the level of the individual cell.
Our system traps the cells in a shear-free environment, allowing us to exchange fluids about the cell to probe with various lectins. By adding the appropriate free sugars to the fluid, the bound lectin can be eluted, and the cells probed with a lectin with another specificity. This ability to probe individual cells and achieve a complete glycoprofile through lectin staining has not been reported before.

1. Cell surface glycosylation

The surface of the mammalian cell has a rich variety of glycan structures bound to most of its various proteins and lipids. These surface displayed glycans can hold much information about the state of the cell and act as signalling molecules for nearby cells and the immune system. Changes in the glycosylation pattern can be used to detect changes in the cell condition, for example, cells undergoing apoptosis display increased amounts of N-acetyl glucosamine (GlcNAc) and mannose.

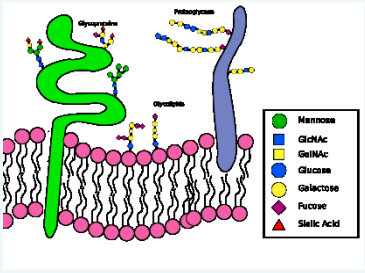


Fig. 1. *Glycans can be attached to lipids or proteins embedded in the cell membrane.*

2. Current methods in glycan characterisation

Glycosylation of proteins can be charaterised using proteomics methods such as LC-MS and MALDI-TOF. Where large amounts of glycoprotein can be isolated, the exact glycan structure can be determined by NMR. These methods require the glycan to be separated from the protein and analysed.
A faster and cheaper method is to use lectins. These are carbohydrate binding proteins which bind specific sugar residues. These have been used for flowcytometry, microscopy and microarray ananlysis. The low binding affinity of some lectins can reduce their usefullness in whole cell analysis and they can be washed off under high-shear conditions.

3. Lab in a Trench

Lab in a Trench (LiaT) is a microfluidic system that can trap cells in a trench by balancing forces of flow and gravity. The cells that are trapped at the bottom of the trench do not experience any shear from the fluid flow above the trench and remain in the locatin where they settled.
Due to the depth of the trench, the flow from the channel over the top of the trench has limited effect on the contents of the trench. At the bottom of the trench, there are shear-free conditions, which is ideal for using low affinity lectins and monitoring cells over time as they do not move. Exchange of solutes in the fluid takes place by diffusion, ensuring flexibility of the system while maintaining shear-free conditions.

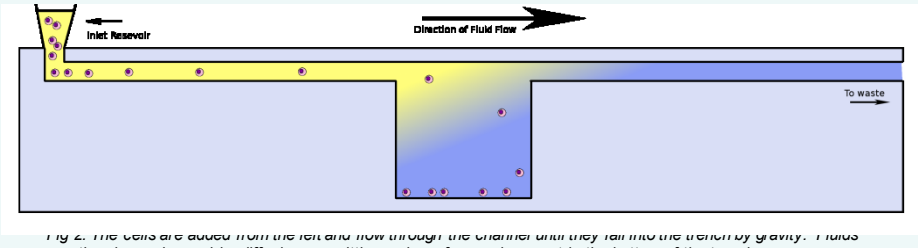


Fig 2. The cells are added from the left and flow through the channel until they fall into the trench by gravity. Fluids can then be exchanged by diffusion, permitting a shear-free environment in the bottom of the trench.

4. Method

Ramos lymphoma cell line was cultured in RPMI 1640 supplemented with 10% foetal calf serum. Biotinylated lectins and DyLight488-streptavidin were purchased from Vector Laboratories.
The trenches are cast in PDMS from an SU-8 master cast on silicon.
TBS buffer with 1mM $CaCl_2$ was used to fill the channels and to dilute lectin and sugars.
Measurements were carried out on an Olympus IX-81 with a heated chamber at 37°C.
Cells were flowed into the system until sufficient cells had been captured as detected by light microscopy. Labelled lectin was then flowed through the system for approximately 15 minutes.
The free sugar corresponding to the lectin was then flowed through the system at a working concentration of 20µM until all labelled lectin had been eluted.
The lectin labelling/elution process was repeated for lectins appropriate to other glycan patterns.

Fig.3 Many trenches can be arrayed on a single disc.

5. Results

Sequential elution of lectins from live cells using free sugars was demonstrated using the LiaT system. Fluorescent intensity was calculated using ImageJ.

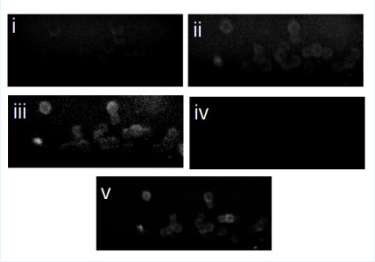


Fig.4 Series of images of sequential elution of fluorophore labelled lectin off Ramos cell. mages I,ii,iii show diffusion of LCA into the channel and staining the cells after 0,5,10minutes.Image iv shows the cells after free mannose has been diffused into the system. Image v is taken after ECL has been added to the system.

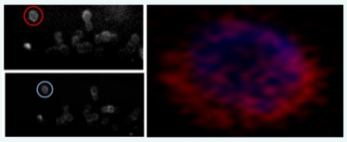
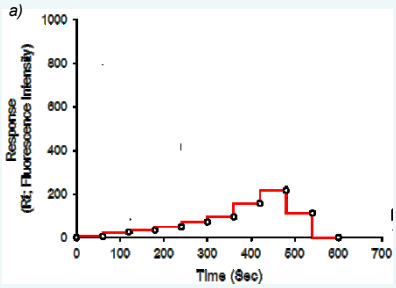


Fig. 5 Overlay image composed of LCA bound to a cell (red circle) and ECL subsequently bound to the same cell (blue circle)



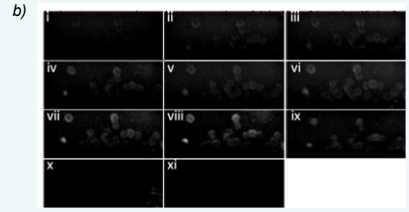


Fig 6. a) Overall fluorescent intensity of the series of images in b as determined using ImageJ.
b) Time series of images taken of LCA binding to Ramos cells (i-ix) followed by elution of the LCA with free mannose (x-xi)

6. Conclusion

This system demonstrates the possibility of sequentially glycoprofiling individual cells in order to fully characterise their surface glycosylation pattern.
LiaT is a powerful alternative to flow cytometry and traditional microscopy, permitting *in situ* visualisation and localisation of various glycan structures on single cells.
The specificity of the lectin binding was demonstrated with the elution of each lectin by its specific free sugar.

7. Acknowledgements

~~~ Here be dragons ~~~
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