FUNCTIONAL CHARACTERIZATION OF DIABETIC CELL MODEL CULTURED ON-CHIP

Patthara Kongsuphol[1,*], Shipi Saha[2], Subhra Kumar Biswas[2] and Qasem Ramadan[1]
[1]Institute of Microelectronics, Agency for Science Technology and Research (A*STAR)
2 Fusionopolis way, #08-02, Innovis, Singapore 138634
[2]Singapore Immunology Network, A*STAR, 8a Biomedical Grove, Immunos, Singapore 138648

* Email: kongsupholp@ime.a-star.edu.sg; Tel.: +65-6770-5607

We developed here a fluidic chip for co-culture of adipocytes and immune cells to mimic *in vivo* environment of diabetes. Type II diabetes is commonly developed as a result of obesity. Evidence suggest that there is excess infiltration and accumulation of immune cells into obese adipose tissue [1-3]. These immune cells release different types of inflammatory cytokine that causes adipose tissue to become chronically inflame which subsequently reduce adipose tissue sensitivity toward insulin as observed in diabetes. Understanding interaction between immune cells and adipocyte as well as pattern of cytokine profile of the adipo-immuno system could lead to insight mechanisms of early diabetes development and possibly early markers of diabetes.

Study of adipo-immuno system in human obesity and diabetes is not well established due to small amount of sample obtained from patients. Current in vitro cell culture often requires large amount of sample for repetitive cell culture and activation making the in vitro method not suitable for human sample study. Alternative system which is able to handle and efficiently utilize small sample is demanded. We develop here a miniaturized adipo-immuno co-culture system to mimic diabetes environment. Adipocyte and immune cells are cultured in separate but fluidically connected compartments with constant supply of nutrient via perfusion (Figure 1). The co-culture cells also fluidically linked to an immunoassay chamber that allows cytokine to be monitored without interfered the co-culture environment.

We first tested fluidic diffusion on chip using colored dye to ensure crosstalk between cells in different compartments (Figure 2) [4]. Once fluidic crosstalk is confirmed, cells were cultured on chip. For co-culture cell model, differentiated human pre-adipocytes were used as adipocytic model whereas peripheral blood mononuclear cell (PBMC) were used as immune cell model. Data indicate that adipocytes can be grown, differentiated and maintained on chip for up to 20 days whereas PBMC could maintain >80% viability on-chip for up to 2 days (Figure 3). PBMC viability on-chip is consistent with PBMC cultured on standard cell cultured plate indicating that the short PBMC lifetime is not due to the on-chip cultured condition but it is the nature of PBMC that could not survive for too long once isolated. Next, glucose uptake experiments were conducted to observe the co-culture system biological activity. LPA was used to induce system inflammation. Result show that adiopo-immuno co-culture with LPA treatment has a tendency to take up more glucose as compared to other groups. Nevertheless no conclusive data can be yet interpreted. More data on insulin treatment and cytokine profile of different treatment conditions are needed.

In conclusion, we have demonstrated a fluidic chip that could be used for adipo-immuno co-culture. Nevertheless, functional study of the chip is still at the early stage and more biological data is needed for study of adipo-immuno interaction.

Acknowledgment
The authors would like the Blood Bank of Health Sciences Authorities, Singapore for kindly providing blood from healthy volunteers for this study. We also thank A*STAR, Singapore for providing support for this project (JCO Grant#1431AFG123).

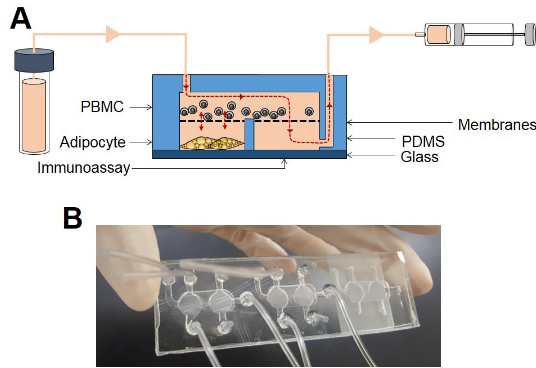

Figure 1*.* *Adipo-immuno co-culture chip. A) Diagram of chip demonstrating adipocyte culture and immunoassay chambers at the lower layer and PBMC culture at the upper layer. Through-membrane perfusion path from upper to lower layer chambers is demonstrated using dashed red line. Glass was used as base and the chip body was fabricated using PDMS sheets. Permeable membrane separates and allows fluidic connection between the two layered chambers. B) Image of the chip in which 2 reactive units can be placed on single glass slide.*

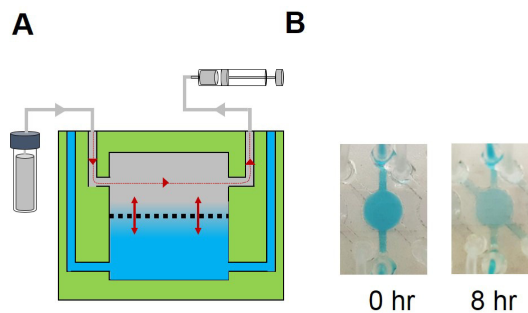

Figure 2*.* *Fluid diffusion test. A) Diagram demonstrates the simple two layered chip design and the perfusion path employed for fluid diffusion test. The lower chip was filled will blue coloured dye DI water whereas the upper chamber was filled with clear DI water. B) Pictures of the two layered chip at 0 and 8 hr of perfusion at 72 µL/hr.*

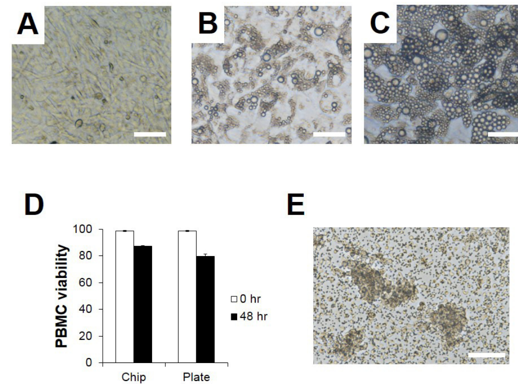

Figure 3*.* *Characterization of adipocytes and PBMC cultured on chip. Pictures of A) human pre-adipocytes, B) adipocyte after differentiation for 7 days and C) adipocytes after differentiation for 14 days. D) PBMC viability cultured on chip or in vitro culture plate at 0 and 48 hr. E) Picture of PBMC cultured on chip. Bar indicates 25 µM.*

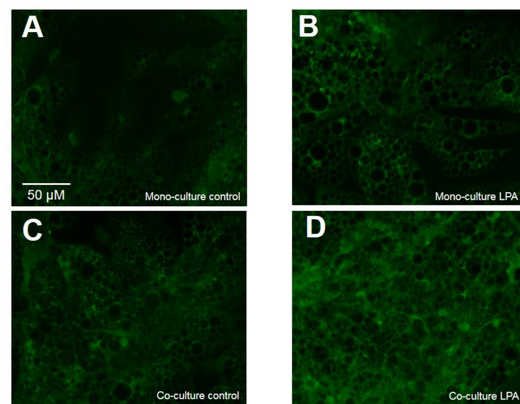

Figure 4*.Glucose uptake assay. Picture of fluorescence signal of glucose uptake in A) adipocyte mono-culture, B) adipocyte mono-culture with 100 nM LPA treatment, C) adipo-immuno co-culture and D) adipo-immuno co-culture with 100 nM LPA treatment.*

REFERENCES:

[1] Hotamisligil, G. S. *Nature* 444, 860 (2006)
[2] Surmi B.K., Hasty B.H. *Future. Lipidol.* 3, 545 (2008)
[3] Weisberg S.P., McCann D., Desai M., Rosenbaum M., Leibel R.L., Ferrante Jr A.W. *J. Clin. Invest*. 12, 1796 (2003)
[4] Kongsuphol P., Liu Y., Ramadan Q., *Biomed. Microdevices.* 18, 93 (2016).