REAL-TIME MEASUREMENT OF CELLULAR REFRACTIVE INDEX AND THICKNESS USING CELL CULTURE CHIP

J. J. Gu[1], Y. F. Yu[1], E. P. Li[2], S. H. Ng[3], P. H. Yap[3], X. Q. Zhou[4] T. H. Cheng[1] and A. Q. Liu[1†]

[1]Nanyang Technological University, Singapore 639798;
[2]Institute of High Performance Computing, Singapore 117528;
[3]DSO National Laboratories, Singapore 117597;
[4]Institute for Infocomm Research, Singapore 117674
([†]Corresponding author. Tel: +65-6790 4336; Email: eaqliu@ntu.edu.sg)

ABSTRACT

This paper reports the measurement of refractive index (RI) and thickness of living cells using cell culture chip integrated with micro-indicators via immersion refractometry. In the experiment, the RI and the thickness of the cultured Madin-Darby canine kidney (MDCK) cell line are obtained simultaneously. As this method features high accuracy, no damage to a group of the living cells and real time measurement during long-term cell culture, it is promising for many applications such as detecting pathological changes of cells and cancer cell detection.

KEYWORDS cell culture, refractive index, immersion refractometry, lab-on-a-chip.

INTRODUCTION

Cellular RI is a significant biophysical property which can be used for many applications such as the evaluation of the culture state [1] and the detection of the pathological change [2]. Performing the immersion method on a microfluidic chip to obtain the cellular RI features high speed and high accuracy as compared with traditional methods. However, the undesirable osmotic pressure change in the cells during measurements still exists. In this paper, a cell culture chip integrated with micro-indicators for the measurement of cellular RI and thickness via immersion refractometry is presented, which minimize the damage to living cells, simplify the manipulation and also be able to perform real time measurement during long-term cell culture.

WORKING PRINCIPLE

Figure 1 shows the working principles of the indicator-based immersion refractometry. The cultured cells were observed together with several indicators, which are of different RI, and consequently different intensity under the phase contrast microscope. The phase shift through the cell in two different media with RI of n_{med} and n_{med}' can be obtained, referring to that of the indicator which has the same intensity as the cell. It is based on the principles that when the cell appears to have same intensities as the ith indicator in the field of vision, they will have the same optical path.

978-0-9798064-1-4/µTAS2008/$20©2008CBMS

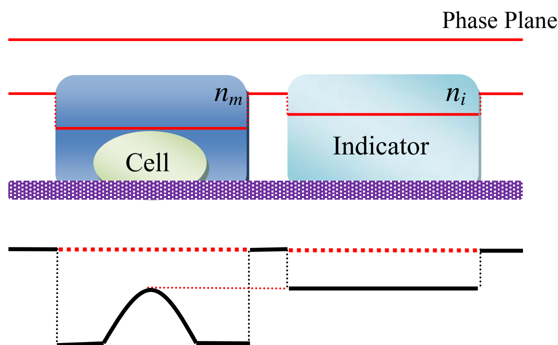

Figure 1. Schematic representation of phase shift through cell and indicator

As such, the solution of two simultaneous equations

$$\begin{cases} \left(n_{cell} - n_{med}\right)\times t_{cell} = \left(n_i - n_{med}\right)\times t_{channel} \\ \left(n_{cell} - n'_{med}\right)\times t_{cell} = \left(n'_i - n'_{med}\right)\times t_{channel} \end{cases}$$

gives both n_{cell} and t_{cell}, where n represents RI and t denotes thickness, with the other parameters already known.

EXPERIMENTAL

Figure 2(a) illustrates the schematic diagram of the biochip. It consists of a cell culture chamber together with 8 indicators in the outer portion. The central chamber with a diameter of 800 µm is surrounded by 5 µm-wide perfusion channels, which allows a uniform distribution of the medium inside. With only one channel thoroughly open, the cell loading process becomes much easier as compared to other's work [3]. Medium refreshing and trypsinization process are manipulated in the opposite direction from the perfusion inlet.

Figure 2(b) shows the whole packaged biochip. PDMS microchip, bonded with poly-L-Lysine coated glass slide, is packaged using a PMMA chip holder to prevent

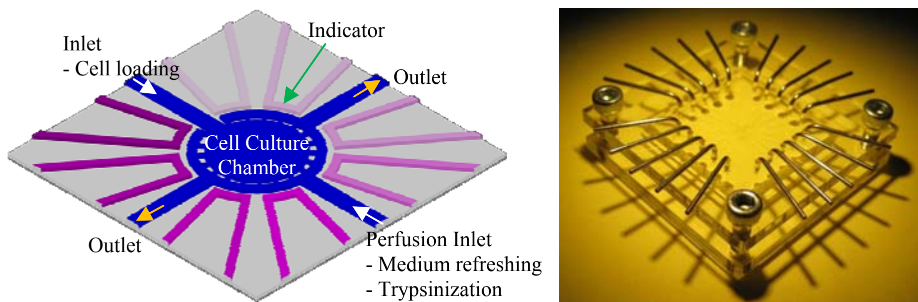

Figure 2. (a) Schematic diagram of the biochip; (b) Photograph of the biochip

the leakage during long-term manipulations. Culturing of MDCK cell line was performed on the biochip. The cell growth was monitored and the images were taken.

RESULTS AND DISCUSSIONS

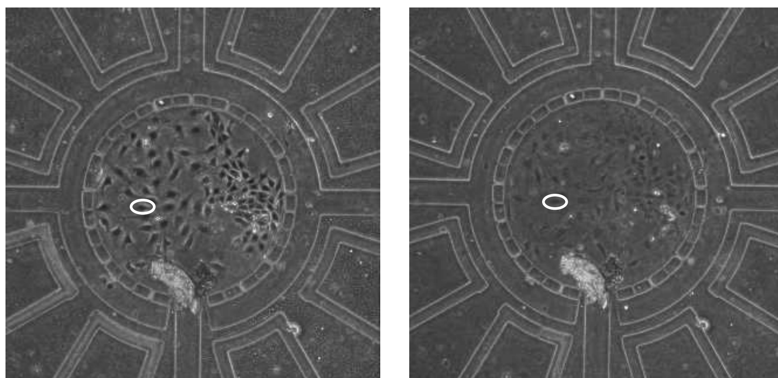

Figure 3. MDCK cells grown on chip surrounded by tunable indicators immersed in (a) DMEM + 10% FBS (RI = 1.3370); (b) BSA solution (RI = 1.3566).

Figure 3 shows the cultured MDCK cells immersed in DMEM + 10% FBS (RI = 1.3370) and bovine serum albumin (BSA) solution (RI = 1.3566), surrounded by 8 indicators filled with various concentration of glycerin-water solution. Normalized intensity of the illustrational cell, highlighted in white circle, and each indicator in two groups are analyzed using MATLAB. Based on the rationale discussed above, RI and thickness of the cell can be obtained i.e. 1.381 and 8.455μm respectively.

CONCLUSIONS

In summary, the cell culture chip is designed, fabricated and experimented for the dynamic measurements of living cell's RI and thickness through the indicator-based immersion refractometry. This method can be utilized for real time monitoring and measurement of cellular physical properties to detect the pathological changes of cells. It is therefore of high potential for cancer cell analysis.

REFERENCES

[1] C. L. Curl, C. J. Bellair, T. Harris, B. E. Allman, P. J. Harris, A. G. Stewart, A. Roberts, K. A. Nugent, and L. M. D. Delbridge, *Refractive index measurement in viable cells using quantitative phase-amplitude microscopy and confocal microscopy*, Cytometry Part A, **65A**, pp. 88-92, (2005).

[2] V. Backman, M. B. Wallace, L. T. Perelman, J. T. Arendt, R. Gurjar, M. G. Müller, Q. Zhang, G. Zonios, E. Kline, T. McGillican, S. Shapshay, T. Valdez, K. Badizadegan, J. M. Crawford, M. Fitzmaurice, S. Kabani, H. S. Levin, M. Seiler, R. R. Dasari, I. Itzkan, J. Van Dam & M. S. Feld, *Detection of preinvasive cancer cells*, Nature, **406**, pp. 35-36, (2000).

[3] P. J. Hung, P. J. Lee, P. Sabounchi, N. Aghdam, R. Lin and L. P. Lee, A *novel high aspect ratio microfluidic design to provide a stable and uniform microenvironment for cell growth in a high throughput mammalian cell culture array*, Lab Chip, **5**, pp. 44-48, (2005).