Assessing in vitro cytotoxicity of cell micromotion by Hilbert-Huang Transform

Yi-Ting Lai -*IEEE Student Membe*r, and Chun-Min Lo

***Abstract*—Electric cell-substrate impedance sensing (ECIS) is a powerful instrument for quantifying cell behavior in tissue culture. As cells attach and spread on the sensing electrode, they restrict the current flow and hence cause the increase of electrical impedance. Furthermore, cell motion may reveal itself as electrical fluctuations, which are always associated with living cells and continue even when the cells become fully confluent. The impedance fluctuation is attributed to incessant changes in the size of the cell-substrate space as cells persistently rearrange their cell-substrate adhesion sites. The magnitude of this sort of vertical motion detected by ECIS is of the order of nanometers and referred to as micromotion. In this study, Hilbert-Huang Transform was used as a micromotion analysis tool to distinguish the in vitro cytotoxicity of human umbilical vein endothelial cells (HUVECs) exposed to low levels of cytochalasin B. Hilbert-Huang Transform consists of two procedures: the empirical mode decomposition (EMD) and the Hilbert Transform. The measured impedance fluctuations due to cell micromotion were decomposed into several intrinsic mode functions (IMFs) by EMD, and then these IMFs were transferred to instantaneous frequencies by Hilbert Transform. Both amplitude and phase of instantaneous frequencies were expressed as a time-frequency spectrum, called Hilbert spectrum, which displayed different distribution pattern in response to different cytochalasin B concentration. The total instantaneous energy (IE) of each IMF was also calculated to quantify the spectral difference. In addition to the observation of a dose-dependent relationship, the IE value of the first IMF at 0.1 μM decreased to about 48% of the control value and significantly distinguished the cytotoxic effect of 0.1 μM of cytochalasin B ($P<0.05$).**

I. Introduction

Electric cell-substrate impedance sensing (ECIS) is an impedance-based method to quantify cell behavior in tissue culture. The basic concept of ECIS is that small gold electrodes are fabricated on plastic substrata and culture medium is used as the electrolyte. An approximate constant current source applies an AC signal between a small electrode and a large counter electrode, while the voltage is monitored with a lock-in amplifier. As cells spread and attach on the working electrode, they effectively block the current flow and contribute the overall impedance increase [1]. Additionally, if different frequencies are employed, the amount of current flowing in the spaces between the ventral side of the cell and the substratum, and thus the impedance, will vary [2-4]. To date ECIS has been used in many areas of biomedical research including: the dynamics of cell attachment and spreading [5-7], cell migration [8-10], in vitro toxicology [11-14], and the barrier function of endothelial and epithelial layers [7, 11, 15]. The small changes in the cell-electrode interaction due to cell micromotion cause the measured impedance to fluctuate with time [2]. This allows continuous observation of the dynamics of the action of a toxic agent and of the possible reversal of toxic effects at low concentrations. These micromotion signals are fractal in the time domain [16]. Techniques such as Hurst exponent and de-trended fluctuation analysis (DFA) have been used to evaluate the long-term correlation of the cell-generated fluctuations and distinguish the small difference between cancerous and noncancerous cell lines [17]. In this study, we applied Hilbert-Huang Transform (HHT) to characterize and estimate the cytotoxic micromotion of human umbilical vein endothelial cells (HUVECs) treated with varying concentration of cytochalasin B.

HHT is a nonlinear and non-stationary analysis consisted of empirical mode decomposition (EMD) and Hilbert Transform. The EMD extracts meaningful symmetric and local zero-mean oscillations called intrinsic mode functions (IMFs) and the Hilbert spectrum provides instantaneous frequency in order to obtain more detail information for time-frequency analysis [18]. Nowadays, Hilbert-Huang transform is a powerful tool for nonlinear and non-stationary signal analysis and is widely applied in structural damage [19], geography [20-22] and biomedicine [23-25].

II. Materials and Methods

Cell culture procedures and the ECIS measurement of micromotion were described previously [11]. In this section, we focus on the micromotion analysis using Hilbert-Huang Transform. When 4000 Hz frequency of AC signal is applied for the impedance measurement of the cell-covered electrode, the fluctuations in the resistance are much larger than those in the capacitive reactance. In this situation, the resistance time series predominates and reveals the optimal observation of the activities of HUVECs. Thus, we only analyzed the resistance time series for distinguishing the cytotoxic effect on cell micromotion.

For detection of cell micromotion, the resistance time series was normalized to the average value of each data set. The normalized time-series data were decomposed by EMD. EMD is recursive decomposition by the original signal subtracting its mean envelope until two stoppage criteria are satisfied: first, the number of extrema and zero-crossing must be the same or differ at one. Secondly, the mean envelope is contributed by the envelope of local maximum and minimum. This process is also called sifting. After the first IMF is

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Yi-Ting Lai and Chun-Min Lo are with the department of biomedical engineering, National Yang-Ming University, Taipei, Taiwan (corresponding author to provide e-mail: cmlo@ym.edu.tw).

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extracted, the first IMF is subtracted by the original signal and followed by sifting. Hence, the normalized time-series X(t) will be separated into several IMFs and a residue rn(t) by EMD as shown in (1).

$$X(t) = \sum_{j=1}^{n} IMF_j(t) + r_n(t) \tag{2}$$

Here, the first IMF contains the highest frequency component; other IMFs decrease their frequencies as the number of IMF increases. However, in order to identify a physical meaning of IMFs, IMFs can be computed by Hilbert Transform. Both amplitude and phase of instantaneous frequencies will be presented as a time-frequency spectrum (or Hilbert spectrum) of IMFs. The Hilbert Transform Y(t) is computed by IMFs as

$$Y_j(t) = \frac{1}{\pi} P\int_{-\infty}^{\infty} \frac{IMF_j(\tau)}{t-\tau} d\tau \tag{3}$$

where P is the Cauchy principle value. If IMF(t) and Y(t) are the real part and imaginary part of Hilbert Transform Z(t), it can be rewritten by Euler formula as

$$Z_j(t) = IMF_j(t) + iY_j(t) = A_j(t)e^{i\theta_j(t)} \tag{4}$$

Thus, the amplitude and phase can be defined by the following expression,

$$A_j(t) = \sqrt{IMF_j^{\,2}(t) + Y_j^{\,2}(t)}$$
$$\theta_j(t) = \tan^{-1}(Y_j(t)/IMF_j(t)) \tag{5}$$

The instantaneous frequency can be obtained as

$$\omega_j(t) = d\theta_j(t)/dt \tag{6}$$

Therefore, the Hilbert spectrum were expressed as

$$H(\omega,t) = \sum_{j=1}^{n} A_j(t)e^{i\int \omega_j(t)dt} \tag{7}$$

Marginal Hilbert spectrum can be defined as

$$h(\omega) = \int_0^T H(\omega,t)dt \tag{8}$$

The marginal Hilbert spectrum indicates a measure of total energy contribution from each frequency value. On the contrary, the instantaneous energy distribution, an integration of squaring amplitude of instantaneous frequencies over frequency, measures the energy fluctuation of time.

$$IE(t) = \int_{-\infty}^{\infty} H^2(\omega,t)d\omega \tag{9}$$

In addition, summation of the instantaneous energy distribution over time can be used as a quantitative index which is called the total instantaneous energy (IE) to express total instantaneous energy distribution level of time.

III. Results and Discussion

Cytochalasin B is known to block polymerization of actin filaments, quickly change cellular morphology, and interfere with cell motility. Twenty hours after HUVECs were exposed to different levels of cytochalasin B, dose-dependent decrease of the normalized resistance fluctuations was observed for cytochalasin B treated cells as compared with the control (Fig. 1). These time series fluctuations indicate different aspects of cytotoxic effect on HUVECs. While the two higher concentrations were easily distinguished from the other four lowest, it was difficult to tell the differences among the three lowest concentrations and the control. By looking at the resistance time course shown in Fig. 1, one might suspect that impedance fluctuation analysis could be a possible assessment of cytotoxicity.

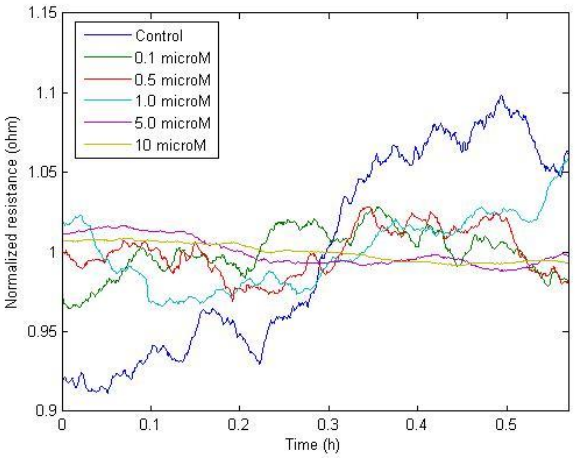

Figure 1. The normalized resistance of HUVEC micromotions 20 h after treated with cytochalsin B in concentrations of 0 (blue), 0.1 (green), 0.5(red), 1.0(cyan), 5.0(magenta) and 10 (yellow) microM.

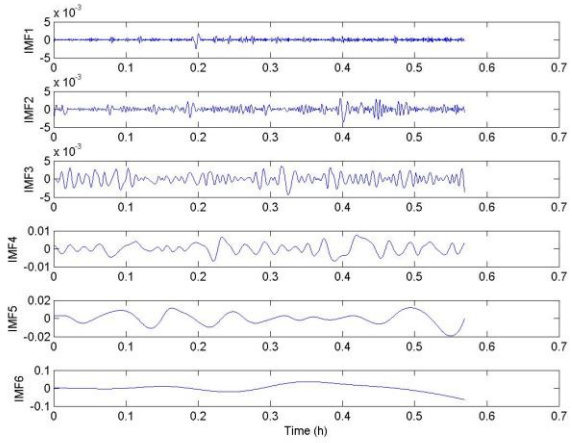

Figure 2. The intrinsic mode functions (IMFs) of normalized resistance in control group.

In previous study, it showed that the power slope from Fourier Transform and Hurst coefficient difficulty distinguish the difference between control and the lower concentration groups (0.1μM, 0.5μM and 1μM)[11]. Here, the normalized resistance time-series were decomposed by EMD and several IMFs and a residue were obtained. By elimination of the residue, IMF was transferred to Hilbert spectrum by Hilbert Transform. Hilbert spectrum, the energy distribution of time and frequency domain, provided multiple dimensional information of micromotion to distinguish cytotoxic effects in response to different concentrations of cytochalasin B. As a result, the Hilbert spectrum of control group indicated that the

most of energy distributed below 0.25 Hz and the main energy focused on the lower frequency area, about under 0.15 Hz.

While the concentration of cytochalasin B increased, the depletion of energy was occurred and the main energy shifted to lower frequency area. In 0.1 μM cytochalasin B group, for example, the energy distributed below 0.2 Hz and the main energy concentrated under 0.1 Hz. For 0.5 μM and higher concentrations of cytochalasin B groups, the energies were mainly contributed at the frequencies below 0.05 Hz.

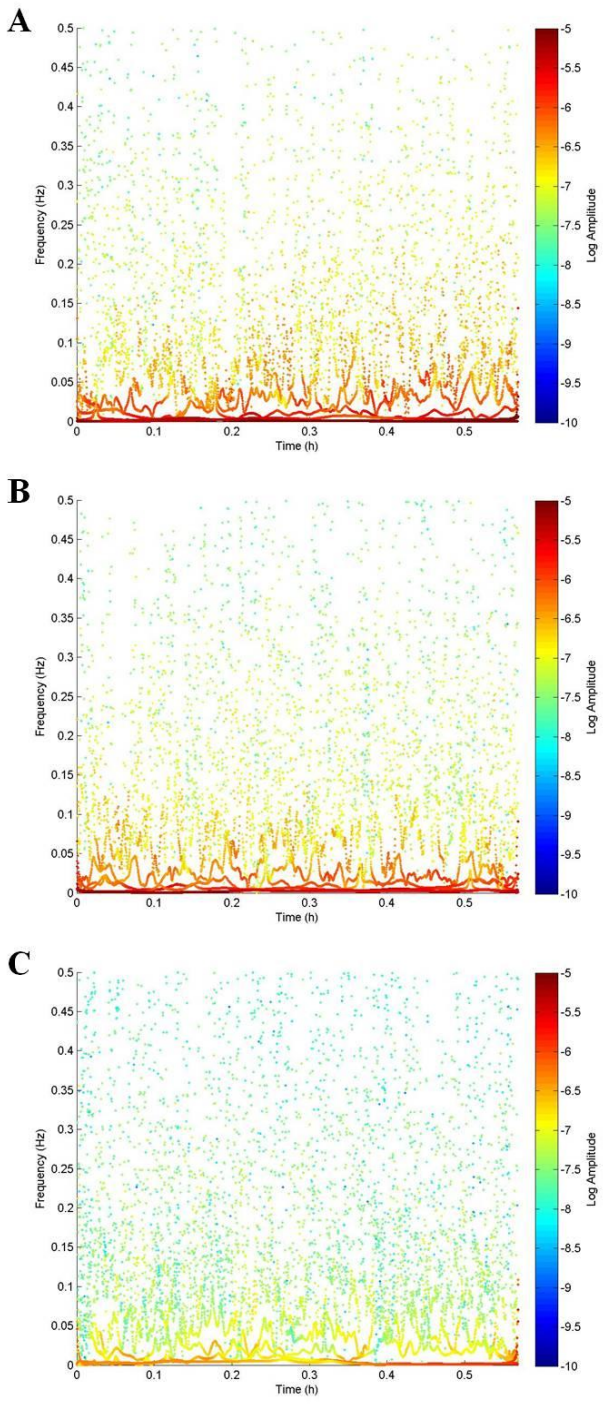

Figure 3. The Hilbert spectrum of HUVECs after 20 h exposed to cytotochalasin B with varying concentrations. (A) Control. (B) 0.1 μM. (C) 5μM.

Although Hilbert spectrum offered the energy distribution of time and frequency to distinguish the toxic effect, it still hardly quantified the differences between lower concentrations of cytochalasin B and the control. Hence, the summation of instantaneous energy distribution over time was quantified as an index, IE, to distinguish toxic levels of cytochalasin B. Table I indicated that IE, which just contained the first IMF component, declined to 48% and 31% of control for cells treated with 0.1 μM and 0.5 μM of cytochalasin B. More significantly, the IE values at all concentrations are separated from their neighboring values and had significant energy depletion compared with the control. The Hilbert-Huang Transform used in this study can be used as an analytical approach for ECIS and other impedance-based biosensors to investigate various aspects of in vitro toxicology.

TABLE I. THE TOTAL INSTANTANEOUS ENERGY (IE) OF HUVECS AFTER 20 HOURS EXPOSED TO CYTOCHALASIN B WITH VARYING CONCENTRATIONS. THE RESULTS WERE NORMALIZED BY EACH CONTROL GROUP.

Conc. (μM)	*IE of IMF1*	*%*	*IE of others IMFs*	*%*
0	6.03×10^{-11}	100	2.30×10^{-7}	100
0.1	2.45×10^{-11}	41*	2.57×10^{-7}	111
0.5	1.69×10^{-11}	28*	1.81×10^{-7}	79
1	1.01×10^{-11}	17*	1.24×10^{-7}	54
2.5	1.06×10^{-12}	2*	8.59×10^{-9}	4*
5	1.82×10^{-12}	3*	5.90×10^{-9}	3*
10	9.05×10^{-13}	1*	1.17×10^{-9}	1*

*: p<0.05, relatively control group

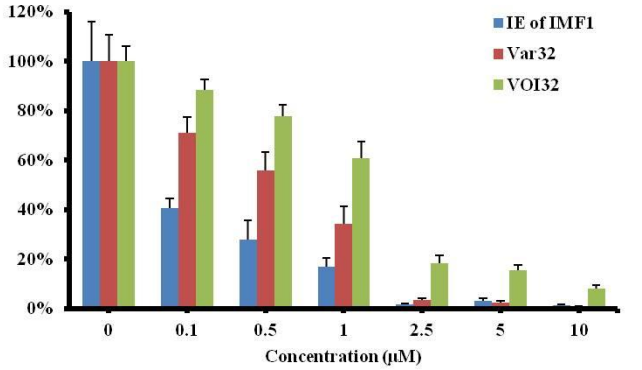

Figure 4. Electrical fluctuation analysis from HUVECs 20 hours were exposure to cytochalasin B. The results were normalized by each control group.

IV. CONCLUSION

We applied Hilbert-Huang Transform to express time-frequency spectrum of micromotion and a quantifiable index, IE, distinguishing the cytotoxicity of HUVECs micromotion after 20 h exposed to cytochalasin B at least 0.1μM.

REFERENCES

[1] I. Giaever and C. R. Keese, "Use of electric fields to monitor the dynamical aspect of cell behavior in tissue culture," Biomedical Engineering, IEEE Transactions on, pp. 242-247, 1986.

[2] I. Giaever and C. R. Keese, "Micromotion of mammalian cells measured electrically," Proceedings of the National Academy of Sciences, vol. 88, pp. 7896-7900, 1991.
[3] C.-M. Lo, C. R. Keese, and I. Giaever, "Impedance analysis of MDCK cells measured by electric cell-substrate impedance sensing," Biophysical journal, vol. 69, pp. 2800-2807, 1995.
[4] C.-M. Lo and J. Ferrier, "Impedance analysis of fibroblastic cell layers measured by electric cell-substrate impedance sensing," Physical Review E, vol. 57, p. 6982, 1998.
[5] I. H. Heijink, S. M. Brandenburg, J. A. Noordhoek, D. S. Postma, D.-J. Slebos, and A. J. van Oosterhout, "Characterisation of cell adhesion in airway epithelial cell types using electric cell–substrate impedance sensing," European Respiratory Journal, vol. 35, pp. 894-903, 2010.
[6] S. E. Klamer, C. G. Kuijk, P. L. Hordijk, C. E. van der Schoot, M. von Lindern, P. B. van Hennik, et al., "BIGH3 modulates adhesion and migration of hematopoietic stem and progenitor cells," Cell adhesion & migration, vol. 7, pp. 405-420, 2013.
[7] S. P. Ngok, R. Geyer, A. Kourtidis, N. Mitin, R. Feathers, C. Der, et al., "TEM4 is a junctional Rho GEF required for cell–cell adhesion, monolayer integrity and barrier function," Journal of cell science, vol. 126, pp. 3271-3277, 2013.
[8] K. R. Schiller, P. J. Maniak, and S. M. O'Grady, "Cystic fibrosis transmembrane conductance regulator is involved in airway epithelial wound repair," American Journal of Physiology-Cell Physiology, vol. 299, pp. C912-C921, 2010.
[9] C.-M. Chan, H.-H. Chang, V.-C. Wang, C.-L. Huang, and C.-F. Hung, "Inhibitory Effects of Resveratrol on PDGF-BB-Induced Retinal Pigment Epithelial Cell Migration via PDGFRβ, PI3K/Akt and MAPK pathways," PloS one, vol. 8, p. e56819, 2013.
[10] J.-A. Ju, C.-T. Huang, S.-H. Lan, T.-H. Wang, P.-C. Lin, J.-C. Lee, et al., "Characterization of a colorectal cancer migration and autophagy-related microRNA miR-338-5p and its target gene PIK3C3," Biomarkers and Genomic Medicine, vol. 5, pp. 74-78, 2013.
[11] D. Opp, B. Wafula, J. Lim, E. Huang, J.-C. Lo, and C.-M. Lo, "Use of electric cell–substrate impedance sensing to assess in vitro cytotoxicity," Biosensors and Bioelectronics, vol. 24, pp. 2625-2629, 2009.
[12] R. Pradhan, S. Rajput, M. Mandal, A. Mitra, and S. Das, "Frequency dependent impedimetric cytotoxic evaluation of anticancer drug on breast cancer cell," Biosensors and Bioelectronics, vol. 55, pp. 44-50, 2014.
[13] R. Pradhan, M. Mandal, A. Mitra, and S. Das, "Monitoring cellular activities of cancer cells using impedance sensing devices," Sensors and Actuators B: Chemical, vol. 193, pp. 478-483, 2014.
[14] K. B. Male, M. Hamzeh, J. Montes, A. C. Leung, and J. H. Luong, "Monitoring of potential cytotoxic and inhibitory effects of titanium dioxide using on-line and non-invasive cell-based impedance spectroscopy," Analytica chimica acta, vol. 777, pp. 78-85, 2013.
[15] A. Singh, R. D. Ramnath, R. R. Foster, E. C. Wylie, V. Fridén, I. Dasgupta, et al., "Reactive oxygen species modulate the barrier function of the human glomerular endothelial glycocalyx," PloS one, vol. 8, p. e55852, 2013.
[16] I. Giaever and C. Keese, "Fractal motion of mammalian cells," Physica D: Nonlinear Phenomena, vol. 38, pp. 128-133, 1989.
[17] D. Lovelady, T. Richmond, A. N. Maggi, C.-M. Lo, and D. Rabson, "Distinguishing cancerous from noncancerous cells through analysis of electrical noise," Physical Review E, vol. 76, p. 041908, 2007.
[18] N. E. Huang, Z. Shen, S. R. Long, M. C. Wu, H. H. Shih, Q. Zheng, et al., "The empirical mode decomposition and the Hilbert spectrum for nonlinear and non-stationary time series analysis," Proceedings of the Royal Society of London. Series A: Mathematical, Physical and Engineering Sciences, vol. 454, pp. 903-995, 1998.
[19] D. Yu, Y. Yang, and J. Cheng, "Application of time–frequency entropy method based on Hilbert–Huang transform to gear fault diagnosis," Measurement, vol. 40, pp. 823-830, 2007.
[20] B. M. Battista, C. Knapp, T. McGee, and V. Goebel, "Application of the empirical mode decomposition and Hilbert-Huang transform to seismic reflection data," Geophysics, vol. 72, pp. H29-H37, 2007.
[21] N. E. Huang and Z. Wu, "A review on Hilbert- Huang transform: Method and its applications to geophysical studies," Reviews of Geophysics, vol. 46, 2008.
[22] Y. Huang, F. G. Schmitt, Z. Lu, and Y. Liu, "Analysis of daily river flow fluctuations using empirical mode decomposition and arbitrary order Hilbert spectral analysis," Journal of Hydrology, vol. 373, pp. 103-111, 2009.
[23] Z. Wang, Q. Zhu, J. Kiely, and R. Luxton, "Hilbert Huang transform impedance measurement data for cellular toxicity monitoring," in Networking, Sensing and Control, 2009. ICNSC'09. International Conference on, IEEE, 2009, pp. 767-772.
[24] H. Li, S. Kwong, L. Yang, D. Huang, and D. Xiao, "Hilbert-Huang transform for analysis of heart rate variability in cardiac health," IEEE/ACM Transactions on Computational Biology and Bioinformatics (TCBB), vol. 8, pp. 1557-1567, 2011.