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Short communication

The xCELLigence system for real-time and label-free analysis of neuronal and dermal cell response to Equine Herpesvirus type 1 infection

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Abstract

Real-time cell electronic sensing (RT-CES) based on impedance measurements is an emerging technology for analyzing the status of cells *in vitro*. It allows label-free, real time monitoring of the biological status of cells. The present study was designed to assess dynamic data on the cell processes during equine herpesvirus type 1 (EHV-1) infection of ED (equine dermal) cells and primary murine neuronal cell culture. We have demonstrated that the xCELLigence system with dynamic monitoring can be used as a rapid diagnostic tool both to analyze cellular behavior and to investigate the effect of viral infection.

Key words: impedance, Cell Index, neuron, EHV-1

Introduction

Monitoring of cell viability is critical to many areas of basic and biomedical research, including virology. In the conventional methods measuring cell proliferation and cell death during viral infection represent the response at a single time point, but the xCELLigence system enables, without the incorporation of labels, continuous measurement and quantification of cell adhesion, proliferation, spreading, cell death and detachment during viral infection (Sari et al. 2009, Witkowski et al. 2010, Slanina et al. 2011). In our previous study we demonstrated that EHV-1 was able to replicate in cultured murine neurons which constitute a good model for investigating interactions between EHV-1 and neuronal cells. We also demonstrated that after infection with EHV-1 some primary cultured murine neurons degenerated but some remained unchanged and survived for more than eight weeks (Cymerys et al. 2010). However, in the case of primary cultured neurons which do not grow as a typical monolayer, microscopic observation of the effects of viral infection is difficult. Therefore, as the xCELLigence system allows continuous and precise

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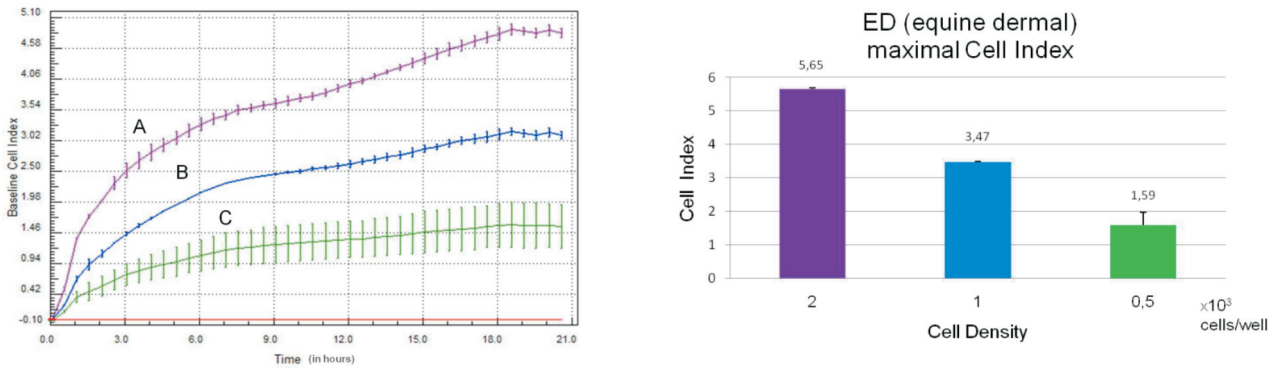

Fig. 1. Influence of ED cell density on impedance measurement. ED cells at a density of (A) $2x10^3$, (B) $1x10^3$, (C) $0.5x10^3$ cells/well. Impedance was measured over 24 h. Bar graph (right) shows the maximal CI.

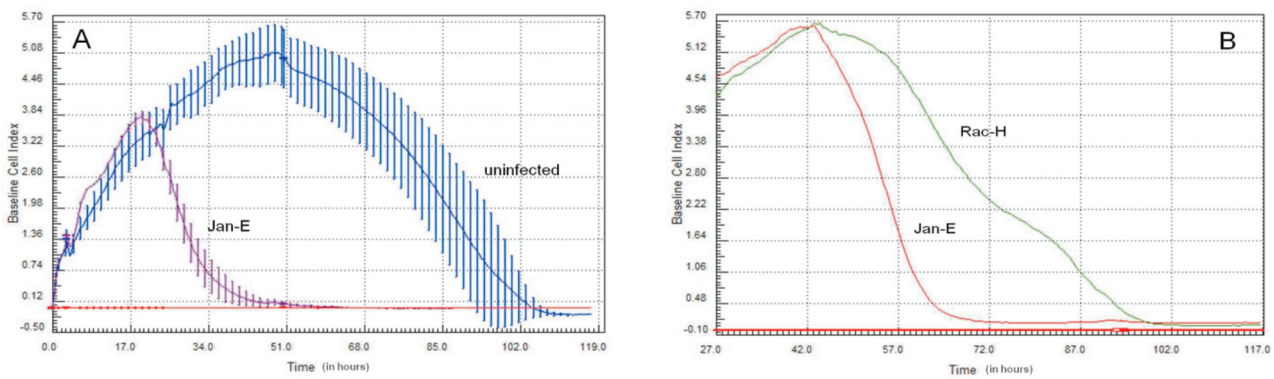

Fig. 2. Influence of ED cell infection with EHV-1. ED cells at a density of $2x10^3$ cells/well. Plot A shows CI values recorded over time in cell culture infected with EHV-1 and uninfected. Plot B shows differences in CI values in cell culture infected with EHV-1 field strain Jan-E and reference strain Rac-H.

measurement and quantification of the cell status, we believe that results obtained using this system would provide an essential complement to the results obtained using traditional research methods.

Materials and Methods

Cell culture and viruses

Primary culture of murine neurons obtained from Balb/c (H-2^d) mice was prepared as described previously (Cymerys et al. 2010). Isolated murine neurons were suspended in B-27 Neuron Plating Medium and adjusted to $2x10^6$, $1x10^6$, $0.5x10^6$ cells/ml. ED cells were suspended in Eagle's minimum essential medium (MEM) and adjusted to $2x10^4$, $1x10^4$, $0.5x10^4$ cells/ml. E-plates were incubated at 37°C with 5% CO_2. Two strains of EHV-1 (10^5 $CCID_{50}$/ml) were used throughout the study: the reference strain Rac-H and field strain Jan-E, originally isolated from an aborted equine fetus.

Cell Index measurement in the xCELLigence system

The xCELLigence system was used according to the manufacturer's instructions (Roche Applied Science and ACEA Biosciences). Cells were seeded in 16-well plates (E-plates16) with a glass bottom coated with capillary gold electrodes. The xCELLigence system measured changes in the impedance which was calculated as a dimensionless parameter called Cell Index. The standard deviation was calculated using RTCA software for each impedance measurement at each time point and for maximal CI values, as showed on the plots.

Results and Discussion

In the present study ED cells at a density of $2x10^3$, $1x10^3$ and $0.5x10^3$ cells/well were seeded in E-plate16 and electrical impedance was measured every 30 minutes. In the case of ED cell culture the highest CI

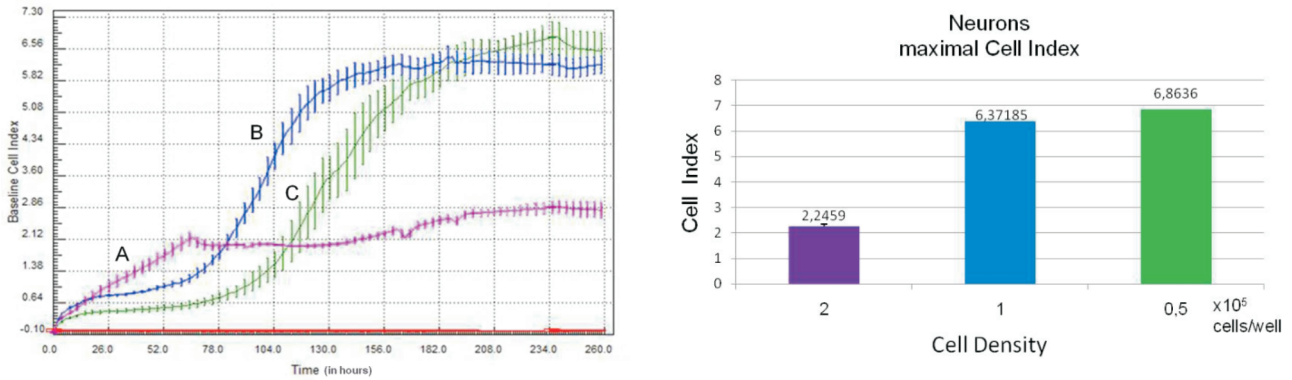

Fig. 3. Influence of primary murine neuron density on impedance measurement. Neurons at a density of (A) $2x10^5$, (B) $1x10^5$, (C) 0.5x105 cells/well. Bar graph (right) shows the maximal CI.

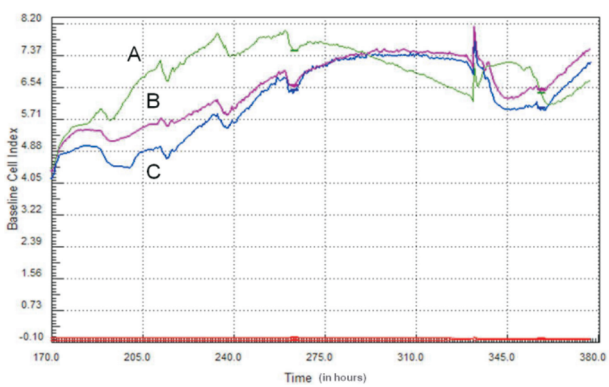

Fig. 4. Influence of primary murine neuron infection with EHV-1 Neurons at a density of $2x10^5$ cells/well were infected with EHV-1 and CI development was monitored. Plot A – uninfected neurons. Plots B and C – neurons infected with EHV-1 (strain Jan-E).

of 5.65 was observed when $2x10^3$ cells/well where seeded (Fig. 1). Neurons were seeded at a density of $2x10^5$, $1x10^5$, $0.5x10^5$ cells/well and, in contrast to the ED cell culture, the highest CI of 6.86 was observed when cells were seeded at the lowest density – $0.5x10^5$ cells/well (Fig. 3). Therefore, the CI increase as well as the time point at which the cell culture reached plateau correlated with the cell number. Afterwards, ED cells were infected at about 4 hours after seeding in a log phase with Jan-E strain of EHV-1. In the second experiment ED cells were infected with Rac-H or Jan-E strain of EHV-1 (Fig. 2). In the case of ED cell culture infected with Jan-E, CI max was reached earlier and was lower than in the case of uninfected ED cell culture. Moreover, a final CI decrease reflecting virus-mediated cytopathic effect and cells detachment at about 46 hours post infection was observed. In the uninfected control the final CI decrease was observed at about 110 hours after seeding (Fig. 2A). We also found that in the case of infection with the Rac-H strain the final CI decrease occurred earlier than in ED cells infected with Jan-E strain (Fig. 2B). In primary murine neuron culture no significant differences in CI value between uninfected cultures and cultures infected with EHV-1 were observed (Fig. 4). In conclusion, our results demonstrate that the xCELLigence system can be used in virological studies to optimize parameters such as cell number, time of infection and virus concentration. Application of this technology will undoubtedly provide a new understanding of the dynamics of EHV-1 infection in different cell types including primary murine neurons.

Acknowledgements

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