ATCC Technology Assessment of Roche xCELLigence™ System — an Electronic Impedance-Based Cell Sensing Unit

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This article describes our systematic investigation into the utility of employing electronic impedance-based cell sensing measurement systems to evaluate changes in cell behavior. We investigated changes resulting from routine passaging (data not shown) and intentional perturbation. Insights gathered from this and other studies present us with new opportunities for introducing advanced levels of cellular characterization and contribute towards development of superior cellular assays at ATCC.

Changes in cellular morphology or growth rate can be early indicators of adverse cellular events. Detection and quantitation of these events with impedance-based cell sensing measurement systems appear to offer improved, real-time, label-free and non-invasive analysis of key cellular events. When adherent cells attach and spread across the sensor surface of an electrode, increases in impedance are recorded. Conversely, cells that round up or detach even for a short time will cause impedance values to drop. One system well equipped to measure cell-impedance is the xCELLigence™ System from Roche Applied Science.

Numerous compounds, including toxins and viruses, are known to induce changes in cell morphology and growth. We explored the ability of the Roche xCELLigence System to assist us in optimizing our current assays and its usefulness in the development of novel assays for the study of both toxins and viruses. Our initial studies were designed to explore the change in impedance following modulation of seeding densities and growth on various extracellular matrix (ECM) coatings. These preliminary experiments enabled us to identify the optimal seeding density and growth conditions for each cell line to use in our virus and toxin studies.

Our first priority was to establish baseline measurements for Madin Darby Canine Kidney epithelial cells [MDCK (ATCC® CCL-34™)] and Green monkey kidney cells [Vero (ATCC® CCL-81™)]. These two cell lines are utilized extensively within the research community and also have industrial applications including use in vaccine production. Figures 1 and 2 illustrate
growth curve data generated from individual wells containing Vero cells (Figure 1) or data plotted as average and standard deviation from replicate wells of MDCK cells (Figure 2). The changes in impedance are expressed as Cell Index (CI) measurement and after reaching a peak level, a decrease in impedance and therefore CI is evident in most of the tracings in Figure 2.

The Real-Time Cell Analyzer (RTCA) software (included with the xCELLigence System) provides an efficient tool to measure key growth parameters, including the calculation of cell-doubling times, and permits the graphical representation of acquired data sets (previously reported in xCELLigence Application Note No. 1 / March 2008). Our experimentally derived doubling times of 13.7 hours and 13.2 hours generated using the xCELLigence System correlated well with the more traditional manual approach. However, the xCELLigence System approach was much less labor and reagent intensive. As an example, Figure 3 was derived from an experimental plan that required measurements from 45 individual flasks. The individual lines labeled A, B and C in Figure 3 represent three different seeding densities. Figure 4 is representative data from the xCELLigence System and shows the replicate curves and the region from which this cell-doubling time was calculated. The 96 well format allowed us
to easily and simultaneously evaluate the doubling time of cells seeded at multiple densities. The inset in Figure 4 illustrates the relative ease with which we can derive the doubling time for each of the six replicate growth curves arising from a single seeding density. Once a correlation is established for a particular cell line (cell impedance versus cell number) the xCELLigence System provides a straightforward process.

We next confirmed the xCELLigence System’s ability to measure early attachment events such as adhesion and spreading as reported previously in an xCELLigence application note (Application Note No. 2 / March 2008). The influence of ECM proteins on adhesion, spreading and morphological characteristics of cultured cells has been well established. Replicate wells were coated with collagen, laminin, and fibronectin respectively. To avoid disruption of cell adhesion and spreading events, all reagents were equilibrated to 37ºC to minimize fluid dynamics due to temperature fluctuations. Measurements were performed every 15 seconds over a 4-hour period in an effort to separate early cell adhesion and spreading from later cell replication events.

Figure 5 reveals trends in the CI tracings that are consistent with the rates of adhesion and degree of spreading observed in similar and parallel studies. Fibronectin induced the greatest overall increase in cell spreading and impedance with collagen (25 µg/mL) showing the next highest CI levels. Dose-dependent changes in impedance were observed in wells with varying concentrations of fibronectin (data not shown), with parallel studies supporting our observations.
that the observed CI changes in these wells were due to morphology changes and not from dramatic differences in the number of adherent cells. However, we did observe differences in the number of cells that adhered to the different ECM proteins with laminin in particular performing sub-optimally in these experiments. Interestingly, the impact of the lower concentrations of ECM proteins on the CI could be mitigated by the presence of 10% fetal bovine serum (data not shown) as serum modulates a number of the same signaling pathways invoked by integrin-mediated cellular binding to ECM proteins.

The impact of viral infection on Vero cell CI profiles was investigated in numerous studies including the comparison of profiles generated by viruses known to induce cytopathic effects (CPE) to those that induce syncytia. Figure 6 is an example of growth profiles from cells infected with a syncytia-forming virus (respiratory syncytial virus, RSV) compared to those infected with a CPE-inducing virus (Dengue virus type 2-New Guinea C, DV). In general, large reductions in CI were seen in most lytic viral infections, and smaller reductions were seen in infections triggering cell-rounding and shrinkage (data not shown). The increase in the CI in the RSV-infected cells in Figure 6 is consistent with cell fusion and enlargement of cells typically seen with syncytia forming infections.

Figure 7 shows a clear dose-dependent effect of Dengue virus infection on cultured Vero cells. Cells were infected with Dengue virus type 2-New Guinea C at a multiplicity of infection (MOI) of 5, 2.5, 1.25 and 0.63 and the data was plotted as a change in CI over the course of the 11-day experiment. All data was
normalized versus the control (uninfected) cells. What is interesting is the apparent CI recovery most evident in the MOI 0.63 tracing. This may be the result of fluctuations in the viral life cycle as it operates in waves. Under certain conditions, cells will recover from one round of infection only to show a drop in viability at a new round of replication.

Another area of particular interest to our organization is the ability of the xCELLigence System to sense previously undetected or subtle changes in cellular behavior. This scenario has presented itself in the form of a “challenging” virus preparation, one that does not reproducibly show clear CPE or syncytia, but demonstrates variable and subtle behaviors seemingly dependant on a combination of temperature, time and host cell line influences. These subtle behaviors have made it increasingly difficult to reproducibly “score” the cellular behavior purely by visual analysis. To investigate this phenomenon more closely, we examined the CI profiles generated in response to cellular infection with a range of virus preparations and MOIs. As expected we observed a variety of CI measurements including both increasing and decreasing CI tracings over time. Figure 8 shows representative tracings generated by viruses prepared at different temperatures and in different cell lines. In this example Vero cells were incubated with virus preparations at an MOI of 5.0. It is evident from this data that both temperature and cell lines have an impact on viral preparations. The ability to investigate numerous conditions in parallel, as well as in real time, has advanced our understanding of the phenomenon and the data generated using the xCELLigence System has initiated further work aimed at gaining more insight into the impact of viral infection on cells. This includes exploring alternative infection parameters towards achieving optimal virus production.

Cytotoxicity is a hallmark effect of a range of reagents including toxins. To optimize our cytotoxicity testing parameters and to extend our toxin and reagent characterization efforts we
explored the xCELLigence System’s usefulness in measuring cellular responses to various toxin preparations, including toxin containing bacterial supernatants and purified toxins. In these studies, replicate wells were seeded with the target cell line and cultures allowed to equilibrate for 24–48 hours prior to being treated with varying concentrations of toxin. Figure 9 shows the impact of several concentrations of purified *Clostridium perfringens* enterotoxin (in the nanomolar to picomolar range) on cell behavior. These experiments were performed using replicate wells seeded with human adenocarcinoma (Caco-2 [ATCC® HTB-37™]) cells. Following 24-hour incubation, the cells were treated with varying concentrations of *Clostridium perfringens* enterotoxin (ATCC® BTX-120) and measurements were taken every 15 minutes over a 24 hour period. CI tracings were normalized to a point prior to addition of toxin and the RTCA software was utilized to calculate the EC$_{50}$ values. Select EC$_{50}$ results were verified using other standard cytotoxicity assays; some of these were significantly more time consuming and labor intensive.

Finally we assessed the system’s ability to rapidly screen for cytotoxicity induced by toxins secreted by various bacterial strains. Figure 10 shows a representative CI tracing induced by a classic toxin producing strain of *Clostridium difficile* as well as profiles from several new strains of *Clostridium difficile* that are being evaluated for distribution by ATCC. Vero cells were seeded onto the plate and permitted to equilibrate and grow for 48 hours prior to the addition of toxin containing supernatant from four different
bacterial strains (strain 1, strain 2, strain 3 and strain 4). The data in this figure was normalized to a point just prior to supernatant addition. Profiles from negative control cells (untreated) and positive control are included as reference. From this image it is clear that strain 1 is quite toxic to the cells while strain 2 and 3 are considered moderately toxic, while strain 4 elicited results that are quite similar to the negative control.

To evaluate toxin specificity we utilized the xCELLigence System to investigate the ability of an inhibitor to block the toxin’s influence on Vero cells (Figure 11). In this figure we are looking at four distinct sets of *Clostridium difficile* supernatants and their response to antisera. Each set of supernatant is represented by a different color and instead of showing thirteen different CI profiles we represented the change in CI as the slope of the curves over a one-hour time frame. In this type of plot the more negative the slope, the more toxinogenic the strain, and conversely the more positive the slope the less toxinogenic the effect. In general, the CI profiles generated from toxin-containing supernatants (Figure 11 samples 1, 4, 7, and 10) were very similar to those shown in Figure 10 in that they showed moderate, strong and minimal to no toxic responses respectively, as indicated by their slopes. In two of the strains, pretreatment with specific antisera was able to mitigate the impact of the toxin on the CI profile (Figure 11 samples 2 and 5). Pretreatment with non-specific antisera had little or no impact on toxicity (Figure 11 samples 3 and 6) and clearly shows the ability of the specific antisera to mitigate the cellular response to the toxin. These results were validated by data generated using a classic cell rounding end point assay, a significantly more time consuming, labor intensive and strictly qualitative end point method. In general, we found that the xCELLigence System allows for rapid, simultaneous cytotoxicity screening of multiple toxin-producing bacteria.
The primary goal of this technical assessment was to explore ways in which impedance-based sensing tools could be used to evaluate changes in cellular behavior. Our studies allowed us to evaluate multiple aspects of the system including sensitivity and flexibility. The xCELLigence System offers dynamic, real-time, label-free and non-invasive analysis of a variety of cellular events. In some scenarios, it offers considerable labor and reagent savings when compared to more classical approaches. Our results demonstrate that the xCELLigence System possesses the speed and sensitivity to measure early changes in cell behavior, such as cell adhesion and spreading, and is able to discriminate dose-dependent changes resulting from virus or toxin addition. The xCELLigence System offers an efficient way to optimize our cell-based assays and provides us with data that is almost impossible to capture using typical endpoint assays. Studies aimed at supporting our preliminary observations and further evaluating this technology for additional screening and characterization applications are ongoing.

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