Technical Overview

Cell Analysis

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Screening and Characterizing Antiviral Drugs in Real Time Using xCELLigence RTCA eSight's Combination of Impedance and Imaging

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Introduction

Using gold biosensors integrated within microtiter plates, the Agilent xCELLigence RTCA eSight uses impedance to continuously and noninvasively monitor changes in cell number, size, attachment strength, and barrier function. Because each of these four parameters is affected over the course of a typical viral cytopathic effect (CPE), impedance is a highly sensitive means of identifying and characterizing compounds that mitigate or block viral CPEs. Positioned adjacent to the gold impedance biosensors in each well, a microscopy viewing window enables eSight to simultaneously monitor viral CPEs using brightfield and fluorescent (red, green, and blue) imaging. Despite having a simple workflow with minimal hands-on time, this information-rich assay provides both a primary result and a confirmatory result from an orthogonal perspective. This technical overview demonstrates the ability of this eSight assay to identify and characterize antiviral drugs using adenovirus as a model system.

Results and discussion

293A host cells, engineered to express blue fluorescent protein in their nuclei, were seeded into eSight's electronic plate at a density of 6,000 cells/well. After 24 hours, adenovirus 5, engineered to express green fluorescent protein (GFP) behind a CMV promoter, was added at a multiplicity of infection of 1 in the presence or absence of different drugs. As shown in Figure 1, over the next 80 hours, untreated cells continue to proliferate up to the point of confluence, causing the impedance signal to steadily increase before plateauing (black data trace). When adenovirus is added in the absence of drug, cells continue to grow for approximately 20 hours before displaying a massive CPE, with the impedance signal dropping to zero by the 65-hour time point (red data trace). Whereas stavudine (50 µM) and ribavirin (20 µM)* have minimal impact on the virus-induced CPE, ganciclovir (50 µM), cidofovir (50 µM), and brincidofovir (1 µM)* mitigate the CPE, causing the impedance signal to look more similar to that of uninfected host cells.

While the health of the cells was being interrogated by impedance, photos were being collected from the same wells. Representative photos from the 75-hour time point demonstrate excellent correlation between the impedance traces and the physical status of the cells (Figure 1). Uninfected cells have grown to confluence and only clear cytoplasm and blue nuclei are visible. Cells treated with virus in the absence of drug express an abundance of

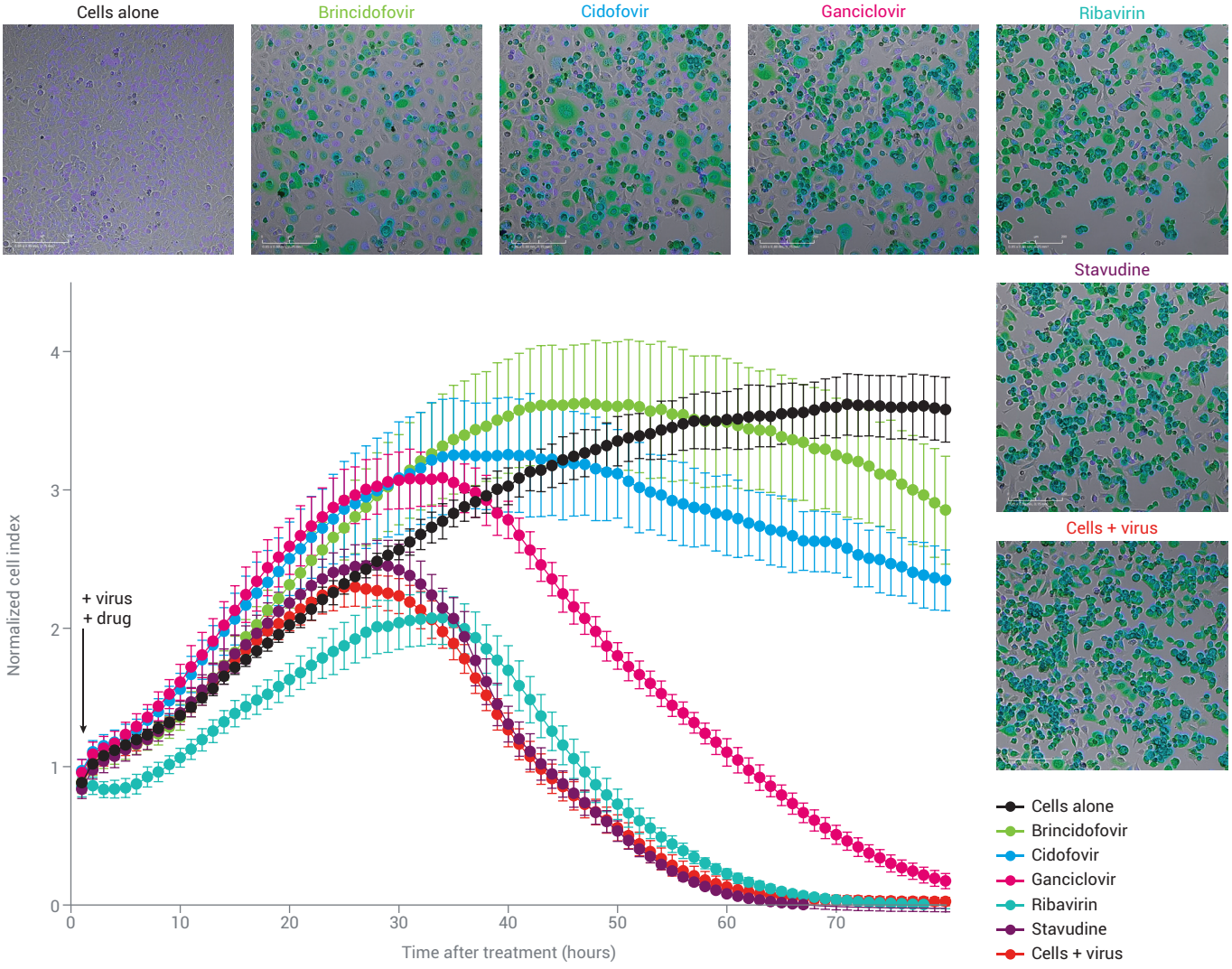

Figure 1. Using both impedance and imaging to screen for drugs that mitigate the adenovirus CPE. Representative photos are from 75 hours post addition of virus and drug. Scale bars = 200 µm.

*The goal was to screen all drugs at a concentration of 50 µM, but this was not possible for ribavirin (because the concentration provided by the manufacturer was too low) or brincidofovir (because of poor solubility).

adenovirus-encoded GFP and display a robust CPE, with cells rounding and detaching from the well bottom. The protection afforded by cidofovir and brincidofovir is clearly evident, with a reduction in the number of cells expressing GFP, a reduction in GFP intensity within individual cells, and a reduction in cell rounding and detachment (Figure 1). Quantitative plots based on imaging parameters such as percent confluence, number of blue nuclei, and GFP intensity all recapitulate the trends observed in the impedance plots of Figure 1 (not shown here due to space limitations).

Having demonstrated the ability of eSight to identify "hits" from a library of drugs, the study next sought to characterize the efficacy of the lead compound, brincidofovir. Progressively increasing the concentration of brincidofovir from 7.8 to 1,000 nM causes a stepwise increase in the impedance signal; the higher the drug concentration, the more cells behave like the uninfected control sample (Figure 2A).

Plotting the area under these impedance traces as a function of brincidofovir concentration yields the dose-response curve in Figure 2B, which has an excellent fit and indicates an EC_{50} of 265 nM.

Within the same wells from which the above impedance data were collected, photos were simultaneously acquired to track the progression of adenovirus-encoded GFP expression. Consistent with its ability to inhibit adenoviral replication[1], brincidofovir suppresses GFP expression in a dose-dependent manner (Figure 2C). Plotting the area under these curves as a function of brincidofovir concentration gives the dose-response curve in Figure 2D, which indicates an EC_{50} of 325 nM. Both the impedance-based and imaging-based dose-response curves would benefit from the inclusion of higher brincidofovir concentrations, but running assays above 1,000 nM was not possible due to the limited solubility of this compound.

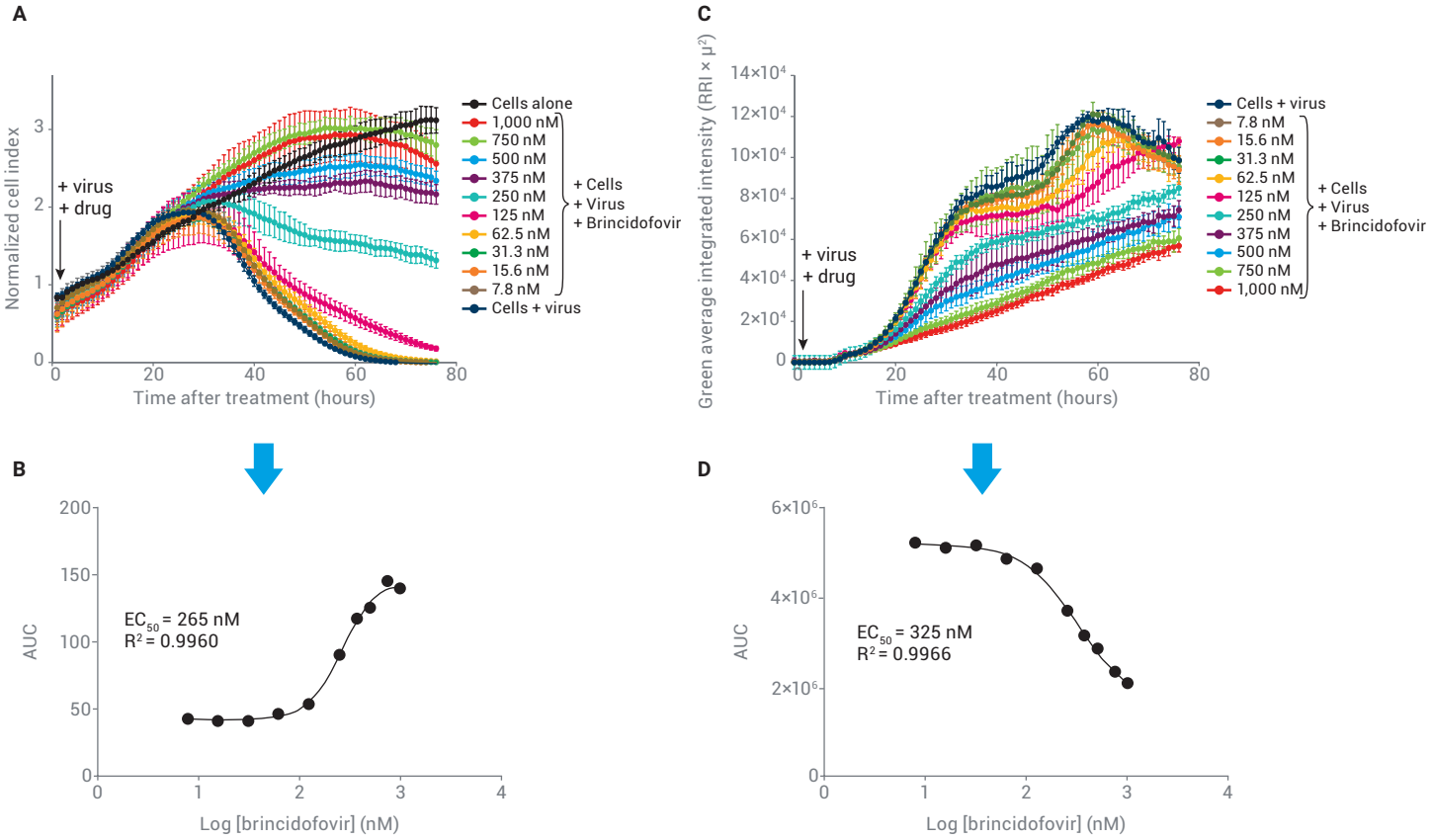

Figure 2. Characterizing the efficacy of brincidofovir against adenovirus using impedance (A). The area under the impedance traces in panel A was plotted as a function of brincidofovir concentration to yield a dose-response curve (B). The efficacy of brincidofovir was also quantified using the average integrated intensity of the adenovirus GFP signal (C and D).

Conclusion

Across a broad array of applications (small molecules, neutralizing antibodies, etc.), virology assays typically monitor changes in the infected host cell over time. The traditional methods for quantifying these virus-induced CPEs are often labor-intensive and only yield endpoint data. In contrast, the eSight assay described in this study only requires a cell-seeding step and the subsequent addition of virus with or without drug. No additional handling or processing steps are required. The continuous nature of the impedance and imaging data make it possible to track the full continuum of the viral CPE, which in turn enables one to focus analyses on optimal time windows. This stands in stark contrast to endpoint assays that only provide a "snapshot" of the CPE, and only do so at a small number of arbitrary time points.

Although a gold standard in science has historically been to confirm results using an alternative technique, this is becoming increasingly less common due to time and financial constraints. Without increasing the amount of hands-on time required, eSight provides a primary and confirmatory result simultaneously. The close agreement between the impedance-based and imaging-based EC_{50} values reported in this technical overview is typical for eSight assays and reflects the fact that these orthogonal analysis methods are interrogating the same population of cells at the exact same time.

Note that the imaging-based readout used in this study identifies effective drugs by their ability to reduce adenoviral GFP expression. This "loss of signal" format is not ideal for drug screening because one cannot immediately differentiate between: i) loss of GFP signal due to *bona fide* inhibition of the virus, or ii) loss of GFP signal due to a nonspecific inhibition of the cell itself. In contrast, the impedance-based readout used in this study identifies effective drugs by their ability to effect a gain in signal. The only way this can happen is if the drug both inhibits the virus and does not inhibit the host cell. This is one reason to use impedance as the primary readout and using the imaging data in a confirmatory role.

In summary, the eSight assay described in this technical overview serves as an efficient means of both identifying and characterizing antiviral compounds. The assay format is flexible, extending well beyond what was used here, and has been broadly adopted globally in studies of diverse viruses (enveloped, nonenveloped, dsDNA, ssRNA, etc.) and host cell types.

Reference

1. Alvarez-Cardona, J. J. *et al.* Brincidofovir: Understanding its Unique Profile and Potential Role Against Adenovirus and Other Viral Infections. *Future Microbiol.* **2020**, April, *15*, 389–400.

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