Introduction
Most conventional cell-based in vitro assays for the assessment of cell viability and cytotoxicity are disruptive endpoint assays requiring cell lysis. A non-invasive and label-free way to continuously monitor cellular behavior can be achieved using the xCELLigence System Real-Time Cell Analyzers (RTCA) co-developed by Roche and ACEA Biosciences (1). RTCA Instruments measure changes in electrical impedance when adherent cells contact microelectrode sensors at the bottom of each well of the dedicated E-Plate cell culture plate. This technology allows long-term, real-time dynamic monitoring of cellular events, such as cell adhesion, proliferation, cell death, spreading, and other morphological alterations. In addition, the combination of continuous monitoring with downstream applications, such as biochemical assays, proteomics, and genomics, (2) is a powerful way to carry out high-throughput predictive toxicological assessments (4).

Each of the 96 wells of the standard E-Plate 96 contains integral sensor electrode arrays that cover approximately 80% of the well bottom. This high level of electrode coverage allows researchers to take uniform cell behavior measurements across the entire well. The newly developed E-Plate VIEW 96 uses a new microelectrode array design that incorporates a 0.5 mm wide transparent window in each well, making it possible to visually inspect the well along its entire diameter (see Figure 3A). This allows researchers to observe measured changes using microscopes and automated cell imaging systems; in addition, this configuration also combines visual inspection with real-time, impedance-based monitoring of cell behavior – all in a single 96-well plate.

In the present study, we assessed cytotoxicity of doxorubicin-treated CHO-K1 cells with the xCELLigence System and, in parallel, using the high-throughput imager Cellavista System (Roche). Doxorubicin, a commonly used chemotherapeutic agent, belongs to the class of drugs called anthracyclines. Doxorubicin-induced cytotoxicity was continuously monitored using the E-Plate VIEW 96 with an RTCA MP Instrument, generating a continuous compound- and concentration-dependent Cell Index (CI) profile. In specified time intervals, cell morphology was also monitored on the E-Plate VIEW 96 by automated image acquisition using the Cellavista System.

Our findings show that continuous monitoring using the xCELLigence System provides detailed information about cell culture conditions throughout the time course of the experiment. In addition, the microscopic images of cultured cells at different time points following compound administration accurately reflect the onset and progression of doxorubicin-induced cell death. This basic workflow
application (see Figure 1), including cell counting using the Cedex XS Analyzer, real-time cell analysis using the xCELLigence System, and high-throughput imaging using the Cellavista System, underscores the added value of the E-Plate VIEW 96 for toxicology, pharmacology, and cell biology.

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Materials and Methods

Cell culture

Chinese hamster ovary (CHO-K1) cells (ATCC) were plated in Ham’s F12 cell culture medium, supplemented with 10% FCS and 2 mM glutamine, and cultured at +37°C in a 5% CO2 humidified atmosphere. Cells were harvested by trypsinization, washed in cell culture medium, and plated at 20,000 cells per well of an E-Plate VIEW 96. Prior to plating, cell concentration, aggregation rate, and viability were assessed by automated trypan blue exclusion using the Cedex XS Analyzer (Roche). To increase statistical accuracy, measurements were performed in duplicate on a Cedex Smart Slide.

Monitoring of cell growth using the RTCA MP Instrument

Cell growth was continuously monitored for 78 hours using the RTCA MP Instrument. Background impedance was measured in 100 µl cell culture medium per well. The final volume was adjusted to 200 µl cell culture medium, including 2 x 10⁴ CHO-K1 cells per well. After plating, impedance was measured according to the following schedule: 1 minute intervals for 2 hours, 5 minute intervals for 2 hours, and 15 minute intervals until compound treatment. Twenty hours post-plating, cells were treated with different concentrations of doxorubicin (375 nM to 100 µM). After compound administration, impedance was measured in 1 minute intervals for 2 hours, 5 minute intervals for 2 hours, and 15 minute intervals for the remaining time. All experiments were performed in triplicate. Cell Index (CI) values were normalized to the time point of compound administration, referred to as normalized CI. The cytotoxic effect of doxorubicin was quantified by calculating an IC₅₀ value using the RTCA Software 1.2.1.

Automated cell imaging using the Cellavista System

High-throughput brightfield microscopy was performed at specified time points throughout the time course of the experiment using the Cellavista System. For this purpose, xCELLigence System measurements were briefly paused, and the E-Plate VIEW 96 was transferred to the Cellavista System. Brightfield images were acquired from all 96 wells using the 20x objective, which requires only 7 images to cover the entire E-Plate VIEW well. As indicated in Figure 3C, whole-well image acquisition of an E-Plate VIEW 96 by the Cellavista System is accomplished using only 1, 2, 4, or 7 images per well using either the 2x, 4x, 10x, or 20x objective, respectively. After image acquisition, the E-Plate VIEW 96 was returned to the RTCA MP Instrument and impedance measurement was resumed.
Figure 2: Cell counting and cell viability evaluation. (A) Before plating CHO-K1 cells on the E-Plate VIEW 96, total cell number and cell viability were assessed by trypan blue exclusion using the image-based Cedex XS Analyzer. (B) The resulting histogram shows the diameters of all detected objects, including viable and dead cells, aggregates, and debris.

<table>
<thead>
<tr>
<th>Objects</th>
<th>Diameter [µm]</th>
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<tbody>
<tr>
<td>debris</td>
<td></td>
</tr>
<tr>
<td>single cells</td>
<td></td>
</tr>
<tr>
<td>cell cluster</td>
<td></td>
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</tbody>
</table>

Number of objects:
- 120
- 100
- 80
- 60
- 40
- 20
- 0

Viability: 96 %

Total cells per ml: 8.3 x10^5
Viable cells per ml: 8.0 x10^5
Dead Cells per ml: 3 x10^4

Figure 3: Image acquisition on E-Plate VIEW 96. (A) Micrographs of the conventional E-Plate 96 and new E-Plate VIEW 96. (B) Full plate overview of an E-Plate VIEW 96. (C) Whole-well image acquisition by the Cellavista System of an E-Plate VIEW 96 is accomplished with 1, 2, 4, or 7 single images using either a 2x, 4x, 10x, or 20x objective, respectively.
Results

Cell culture and real-time cell analysis

Prior to plating of CHO-K1 cells on the E-Plate VIEW 96, cell concentration and cell viability were assessed using the semi-automated image-based Cedex XS Analyzer, based on the Trypan Blue Exclusion Method (see Figure 2). In addition to information on cell viability (in this case 96%) and cell concentration (in this case $8 \times 10^5$ viable cells per ml), the Cedex XS Analyzer produces information about cell size, diameter, morphology, and aggregation rate (see Figure 2B). According to the Cedex XS Analyzer data, $2 \times 10^4$ viable CHO-K1 cells were plated into each well of an E-Plate VIEW 96.

Real-time cell analysis of cultured CHO-K1 cells was performed using the xCELLigence RTCA MP Instrument. As shown in Figure 5B, the Cell Index (CI) profiles reflect the biological status of cultured cells throughout the time course of the experiment. Cells attached during the first 4 hours of cultivation, reaching a CI value of approximately 0.4. Cells were then allowed to adapt to the cell culture conditions during an overnight pre-cultivation, reflected by a logarithmic growth phase 20 hours post-plating. The administration of doxorubicin at 20 hours post-plating resulted in a dose-dependent decrease in CI values, whereas CI values of control cells increased and reached a plateau 70 hours post-plating, indicating cell confluence. The cytotoxic effect of doxorubicin treatment on CHO-K1 cells was quantified by calculating the IC$_{50}$ value, yielding an IC$_{50}$ of 2.1 μM doxorubicin at 42 hours post-treatment (see Figure 5C).

High-throughput image acquisition

Because the monitored impedance profile from the xCELLigence System is mainly generated by cell contact with the microelectrode sensors, we first tested whether cell growth is comparable on the two different substrates, gold and glass, found at the bottom of each well. As shown in Figure 4, an even distribution of CHO-K1 cells could be detected by microscopic inspection of crystal violet-stained cells in each well of the E-Plate VIEW 96, indicating a uniform, homogeneous cell proliferation rate.

To gain further insight into the onset of doxorubicin-induced cell death, CHO-K1 cells were microscopically inspected using the high-throughput Cellavista System. Full plate scans were performed at the time points, specifically indicated by the series of small peaks found in the CI profile, highlighting the sensitivity of the xCELLigence System (see Figure 5B). As an example, close-ups of control and 10 μM doxorubicin-treated cells are shown in Figure 5A, revealing the significant detrimental effect 22 hours after compound administration. This finding is in line with results collected by the xCELLigence System, which show a significant decline of the CI profile at the same time point. This finding shows that the results from the xCELLigence System are correlated to the cellular status at each time point examined, eventually leading to large-scale cell death at 32 to 48 hours post-treatment.
Figure 5: Assessment of doxorubicin-induced cytotoxicity on CHO-K1 cells. CHO-K1 cells were treated with different concentrations of doxorubicin (375 nM to 100 µM), 20 hours post-plating on an E-Plate VIEW 96. (A) Cellular effects were monitored throughout the time course of the experiments using the Cellavista System. Close-ups of 20x micrographs show untreated control and 10 µM doxorubicin-treated CHO-K1 cells 0 to 78 hours after compound-treatment. (B) Cytotoxic effects were continuously monitored using an RTCA MP Instrument. Cell Index (CI) values were normalized to the time point of compound administration. The asterisk (*) symbol indicates time points of image acquisition using the Cellavista System. (C) Doxorubicin cytotoxicity was quantified by calculating the IC_{50} 42 hours post-treatment.
Discussion

Cell-based in vitro assays are essential to cell science research. The xCELLigence System has emerged as a valuable and versatile tool, allowing label-free and real-time monitoring of cell cultures (1, 3). Previous studies described comprehensive workflows, including real-time cell analysis, biochemical assays, qRT-PCR, and microarray analysis (4). However, microscopic applications in conjunction with xCELLigence measurements have so far not been possible. With the introduction of the new E-Plate VIEW 96, we show here, for the first time, simultaneous real-time cell analysis using the xCELLigence System and high-throughput image acquisition performed using the Cellavista System.

Compound-treated CHO-K1 cells were continuously monitored using the xCELLigence System, revealing information in real time about the cell culture conditions. The identified cellular effects, such as cell proliferation and cell death, were verified using high-throughput microscopy, performed in the same E-Plate VIEW 96 used for impedance recordings. The cytotoxic effects of doxorubicin were monitored using the xCELLigence System, resulting in Cell Index (CI) profiles that quantitate the onset and progression of compound-induced cell death. Similar detailed profiles are not possible using imaging applications; however, the complementary microscopic inspection of doxorubicin-treated cells provides additional insight into cytotoxic processes, including cell proliferation and cell death. It is noteworthy that the removal of the E-Plate VIEW 96 from the incubator produced a transient peak in the CI profile, reflecting a minor cellular response to the changing environment. Hence, a fast imaging system, such as the high-throughput Cellavista System, should be used to reduce image acquisition time and to avoid a significant impact on the cultured cells.

Taken together, these experiments emphasize the added value of the E-Plate VIEW 96, allowing real-time cell analysis and cell imaging in each well of the same 96-well plate. Continuous monitoring using the xCELLigence System easily identifies even modest cellular effects, which can be further analyzed by microscopic analysis. Brightfield imaging can be extended to numerous fluorescence-based applications, allowing in-depth analysis of cellular effects using specific biochemical assays.

References

Ordering Information

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The RTCA Analyzer in combination with either the RTCA SP Station or the RTCA MP Station and an RTCA Software Package 1.x, with the E-Plate 96 is a real-time cell based assay system covered by US patent No. 7,192,752 (exp. 11/10/2023), No. 7,470,533 (exp. 11/10/2023), No. 7,560,269 (exp. 10/24/2025).

Intended Use:

For life science research only. Not for use in diagnostic procedures.

Key Words:

Cytotoxicity, E-Plate VIEW 96, cell imaging, Cellavista System, visual inspection, microscopy, xCELLigence System, Cedex XS Analyzer, cell counting, automation