Reuse of E-plate cell sensor arrays in the xCELLigence Real-Time Cell Analyzer

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The xCELLigence Real-Time Cell Analyzer (RTCA) is a non-invasive, impedence-based biosensor system that can measure cell viability, migration, growth, spreading, and proliferation. Changes in cell morphology and behavior are continuously monitored in real time using microelectronics located in the wells of RTCA E-plates. According to the manufacturer's recommendation, E-plates are single-use and disposable. Here, we show that E-plates can be regenerated and reused several times without significantly effecting experimental results.

Most current biological assays are based on the use of labeling reagents followed by optical detection. One alternative to these conventional assays is the xCELLigence Real-Time Cell Analyzer (RTCA) from ACEA Biosciences, a label-free system that integrates molecular and cell biology assays with microelectronics (1,2). This system allows monitoring of cellular processes by measuring the impedance of sensor-electrodes located at the bottom of each well in the RTCA E-plates. Changes in cell number, viability, morphology, and cytoskeletal dynamics are reflected by changes in the signals from the electrodes. The instrument allows non-invasive, real-time monitoring of cells that is highly sensitive, accurate, and produces a large amount of information. Therefore, the RTCA has a broad range of applications in many biological assays, not only for detecting cell adhesion, proliferation, migration, and differentiation (3–5) but also for the detection of cell- and compound-mediated cytotoxicity/apoptosis and receptor-mediated signaling (6–9). The system has been successfully applied in studies of environmental toxicity (10), cellular function (11), anticancer drug candidates (12–16), and microbiology (17–19).

The E-plates used for the RTCA system are single-use; thus, the cost of the plates might limit the number of experiments a researcher can perform. Here, we describe a procedure for regeneration and reuse of RTCA E-plates, and we show that reusing a plate multiple times does not affect the reproducibility of results obtained in cytotoxicity experiments.

Materials and methods

Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), penicillin, streptomycin, L-glutamine, phosphate buffered saline (PBS), 0.25% trypsin-EDTA solution, methanol, 0.1% collagen solution from calf skin, and vinblastine sulfate were purchased from Sigma-Aldrich (St. Louis, MO). Hank's balanced salt solution (HBSS) without phenol red and wheat germ agglutinin (WGA) Alexa Fluor 350 conjugate were obtained from Invitrogen (Carlsbad, CA). Vinblastine sulfate was dissolved in methanol at a concentration of 1 mM.

Cell culture

The human cervical adenocarcinoma cell line HeLa S3 and the human breast adenocarcinoma cell line MCF-7 were obtained from the American Type Culture Collection (ATCC) (Manassas, VA). The cells were cultured in DMEM supplemented with 10% (v/v) FBS, 100 µg/mL of streptomycin, 100 U/mL of penicillin, and 2 mM L-glutamine. Cells were kept at 37°C in a humidified 5% CO_2 incubator.

Real-time cell proliferation monitoring

HeLa and MCF-7 cells were seeded at densities of 2×10^4 and 6×10^4 cells/well, respectively, into an E-plate 16 (ACEA Biosciences, San Diego, CA) containing 100 µL medium per well and monitored on the xCELLigence Real-Time Cell Analyzer Dual Plate (RTCA DP) instrument (ACEA Biosciences). When the cells entered log phase, the vinblastine solution was added to final concentrations of 1.0–20.0 nM. The final methanol concentration in the wells did not exceed 4% (v/v). The cells were treated with vinblastine for 24 h and incubated at 37°C in a 5% CO_2 atmosphere. To calculate the half maximal inhibitory concentration (IC_{50}) values, RTCA software v. 1.2.1 was used. All of the experiments were repeated at least 10 times.

Regeneration of E-plates

The RTCA E-plates were used up to three times in these experiments. After every use of a single plate, the wells were washed two times with PBS. Cells attached to the well bottom were trypsinized by adding 100 µL trypsin-EDTA solution to every well. Next, the cells were removed, and the wells were washed twice with PBS. All of the plate preparation steps were performed in a sterile culture hood. In order to avoid electrode damage, media, buffer, and trypsin were removed by aspiration with blunt plastic pipette tips. Before each re-use

METHOD SUMMARY

The E-plates in the xCELLigence Real-Time Cell Analyzer (RTCA) system can be regenerated and used several times in cytotoxicity studies without losing the reproducibility of experimental data. Here, we present washing and trypsinization steps for reusing the plates.

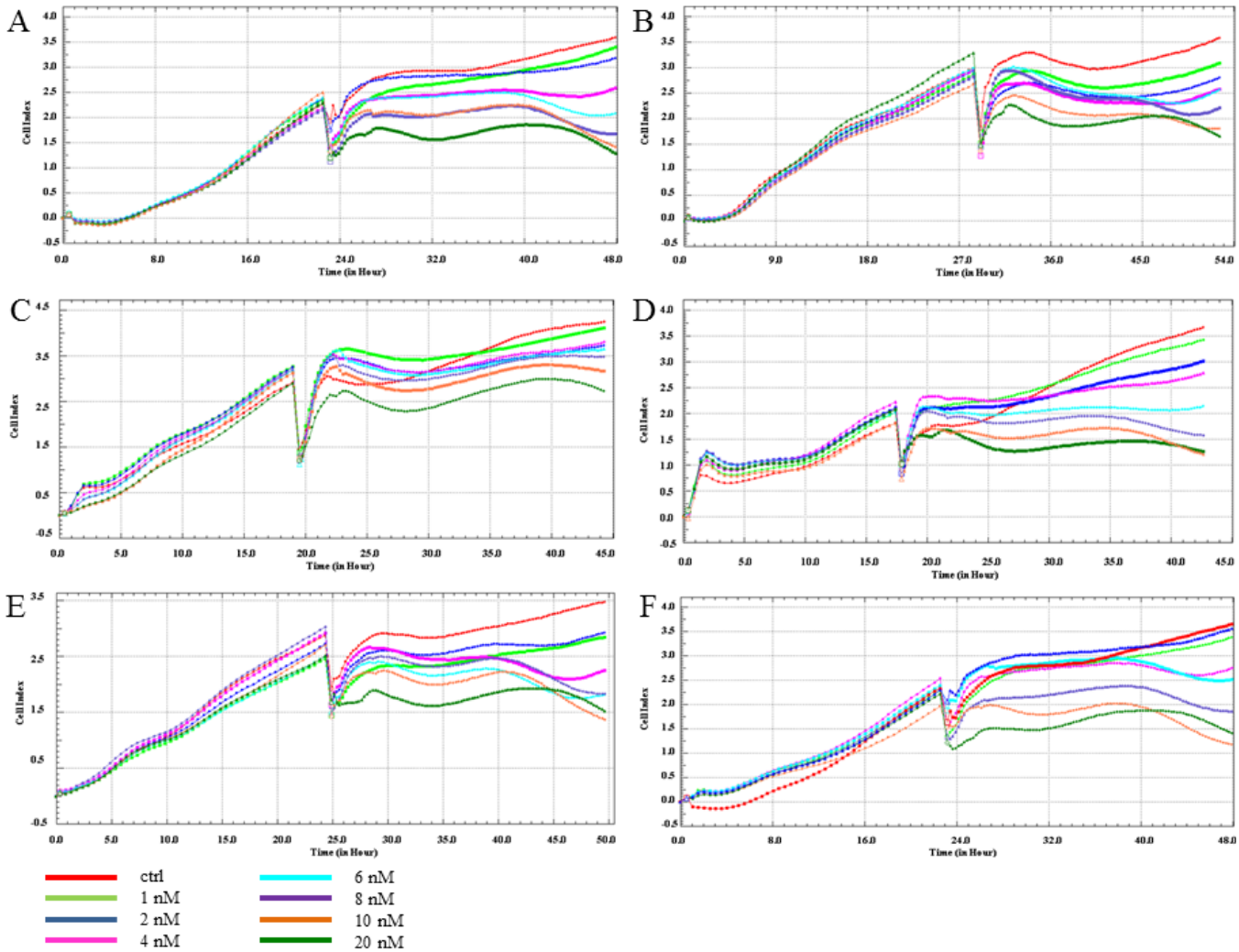

Figure 1. xCELLigence Real-Time Cell Analyzer (RTCA) impedance profiles of E-plates containing HeLa cells treated with vinblastine sulfate for 24 h. Experiments were conducted with new (A,D), once-used (B,E), and twice-used (C,F) E-plates. The plates were either uncoated (A,B,C) or coated with collagen (D,E,F). The different vinblastine sulfate concentrations (1.0, 2.0, 4.0, 6.0, 8.0, 10.0, 20.0 nM) are color-coded. The red line represents the profile of the control sample [cells treated with 4% (v/v) MeOH].

of a plate, we verified the absence of cells at the bottoms of the wells by microscopy. Furthermore, at the start of every experiment, the cell index (CI) background signal (CI signal without cells) for all of the wells was 0 at time point 0, confirming the complete removal of cells during trypsinization.

In the second part of our study, we used E-plates coated with a 0.01% (v/v) collagen solution (final concentration). The bottoms of the wells were coated with 6–10 µg collagen/cm^2. Following protein binding to the well bottom, the excess solution was removed from the wells. The plates were then dried in a culture hood and exposed to UV light for 1 h.

Lectin staining

A fluorescent lectin stain based on a WGA Alexa Fluor 350 conjugate was used according with the manufacturer's protocol. Briefly, the WGA conjugate was prepared as a 1.0 mg/mL stock solution in PBS and then applied to plate wells at a final concentration of 10.0 µg/mL in HBSS buffer. Following a 10 min incubation at 37°C, the wells were washed twice in HBSS and examined under a fluorescent microscope (filter D; excitation 355–425 nm, emission 455 nm) (Leica Microsystems, Heerbrugg, Switzerland). Lectin labeling was performed before and after every trypsinization.

Statistical analysis

All data are expressed as mean ± standard deviation (SD). Statistical comparisons among the results obtained from the experiments were performed using two-way ANOVA. Differences among the data were considered statistically significant if $P < 0.05$.

Results and discussion

To examine the effects of reusing E-plates, we analyzed RTCA impedance profiles and

Table 1. Half maximal inhibitory concentration (IC_{50}) values (nM) for vinblastine sulfate in HeLa cells obtained by the xCELLigence Real-Time Cell Analyzer (RTCA) with E-plates that were used up to three times.

E-plates	IC_{50} [nM]; R^2 [a]		
	First use of plates	Second use of plates	Third use of plates
Plates without collagen	5.06 ± 0.35; R^2 = 0.93	5.22 ± 0.56; R^2 = 0.98	5.11 ± 0.58; R^2 = 0.96
Plates with collagen	5.05 ± 0.37; R^2 = 0.95	5.21 ± 0.50; R^2 = 0.96	5.16 ± 0.39; R^2 = 0.96

[a] R^2: coefficient of determination.
IC_{50} and R^2 results are expressed as mean values obtained from at least 10 independent experiments.

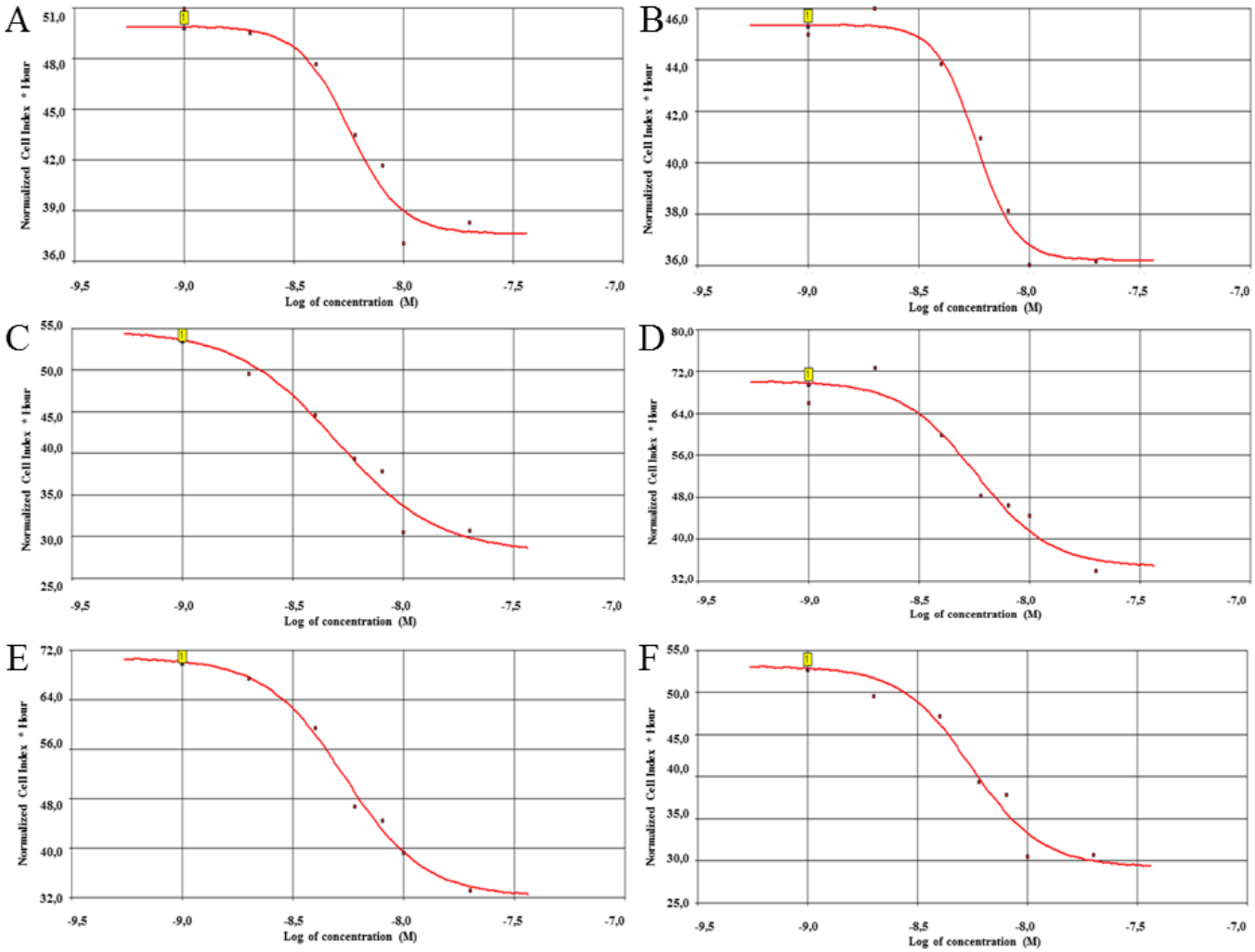

Figure 2. Half maximal inhibitory concentration (IC_{50}) of vinblastine sulfate after 24 h treatment of HeLa cells from 6 different groups of E-plates. For each experiment, IC_{50} values were calculated based on dose-response curves (area under the curve, dose dependent). Experiments were conducted with new (A,D), once-used (B,E), and twice-used (C,F) plates with (D,E,F) or without (A,B,C) collagen coating.

calculated the resulting IC_{50} values for our experiments following vinblastine sulfate treatment of HeLa and MCF-7 cells. We compared the RTCA impedance profiles obtained from six different groups of plates, which were designated as follows: A, B, C—the new, once-used, and twice-used plates, respectively, without collagen coating; and D, E, F—the new, once-used, and twice-used plates, respectively, with collagen coating. Furthermore, in our previous studies using RTCA, we assessed the effect of vinblastine sulfate on the HeLa cells (20).

As shown in Figure 1, the impedance profiles obtained with HeLa cells from all six of the plate groups were comparable. Furthermore, similar results were obtained using MCF-7 cells (Supplementary Figure S1).

For all impedance profiles, the sigmoidal dose-response curves (Figure 2 and Supplementary Figure S2) were obtained, and IC_{50} values were calculated. The calculations were based the CI, which is a relative and dimensionless value representing the impedance change divided by the background value and thus reflects the overall number of cells and the quality of their attachment (11). The CI can change as a function of time, resulting in time-dependent and dose-dependent impedance profiles during an experiment (1,11).

In all experiments, the IC_{50} values of vinblastine sulfate ranged 5.05–5.22 nM for HeLa cells (Table 1) and 8.24–8.39 nM for MCF-7 cells (Supplementary Table S1). Statistical analyses did not show significant differences among the IC_{50} values of the six different plate groups. Hence, neither trypsinization of the cells nor collagen coating influenced the cytotoxicity results.

We used a fluorescent lectin stain that selectively binds to N-acetylglucosamine and N-acetylneuraminic acid residues to examine the efficiency of HeLa cell (Figure 3) and MCF-7 cell (Supplementary Figure S3) trypsinization, as well as assess if residual extracellular matrix was still present on the plate surface. The surfaces of the six different groups of plates were examined before and after each reuse. As shown in Figure 3 and Supplementary Figure S3, after every trypsinization procedure, the surfaces of all tested plates were clean and devoid of extracellular debris.

The xCELLigence system is an innovative technology that allows continuous and quantitative monitoring of cells (11,21). The application of microelectrodes in RTCA plates for the measurement of electronic impedance enables observation of the viability of tested cells at every point of time during an experiment. The data are collected throughout the experiment (1). Additionally, there is no need to terminate the experiment and label cells in order to determine IC_{50} values, which is the case for different end-point assays (22). Furthermore, due to the use of sensor arrays

in the plates, the RTCA system detects cell changes with higher sensitivity than other cytotoxicity assays (23,24) and gives more information than the single-value end-points obtained in classical tests (9,25). Hence, the xCELLigence system provides an advantage in cytotoxicity research, especially for screening potential anticancer drugs and monitoring of the effects of compounds on cells over hours or even days.

However, despite the many advantages of the RTCA system, its main limitation is that the plates are recommended by the manufacturer to be used only once. As a consequence, the cost of repeated experiments is significantly increased.

We showed that the results obtained with reused E-plates are comparable to those from new plates when analyzing the effects of vinblastine sulfate treatment on HeLa and MCF-7 cells. Furthermore, our experiments using collagen-coated plates showed that the use of this substrate did not influence results from new and used plates.

Coating well surfaces with different substrates is mostly done for RTCA experiments involving adherent cell lines. Fibronectin, collagen, or gelatin are used to improve the adhesion, spreading, and growth of cells, especially those with a weaker ability to attach onto the cell culture well bottoms (26). However, Martinez-Serra et al. have also adapted this methodology of pre-coating the cell culture surface with different substrates for use of the xCELLigence system with leukemia/lymphoma suspension cells (27).

We have demonstrated that E-plates containing wells with sensor-electrodes, which have wide application in many research areas, can be regenerated and reused several times. The regeneration of plates reduces the cost of RTCA experiments and, more importantly, did not affect data reproducibility in our experiments. It has to be stressed that the complete removal of the cytotoxic agent after each use is mandatory, although for some compounds this might not be possible. Despite this limitation, our results provide an alternate solution when the cost of new plates is prohibitive (28). Finally, our experiments assayed the effects of the well-known cytostatic drug vinblastine (29), but the protocol can be applied to other cytotoxicity studies.

Author contributions

J.S.H., A.A., and R.B. conceived and designed the experiments. J.S.H. and A.A. performed the experiments. J.S.H., A.A., and R.B. analyzed the data. J.S.H. and A.A. wrote the paper. J.R.O. supervised the study and corrected the manuscript.

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Competing interests

The authors declare no competing interests.

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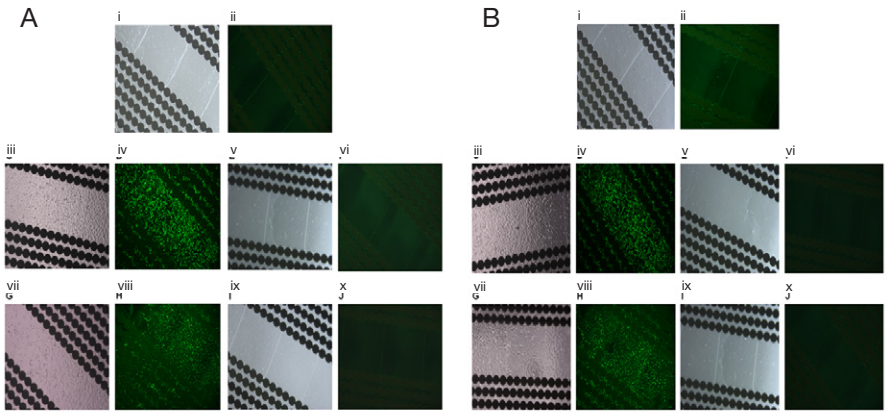

Figure 3. Lectin staining of E-plate surfaces. Plates were either uncoated (A) or coated with collagen (B). Plates were photographed before (i,iii,v,vii,ix) and after (ii,iv,vi,viii,x) staining with WGA Alexa Fluor conjugate. Images iii, iv, vii, and viii show HeLa cells adhered to the plates; images v, vi, ix, and x show plate surfaces after trypsinization. The surfaces of plates (iii–x) without (A) and with collagen coating (B) were compared with those of the new plates (i,ii).

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