The Application of Cell-Based Impedance Technology in Drug Discovery

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ACEA Biosciences

- Founded in early 2002
- Located in San Diego, CA
- Mission: Integration of microelectronics with cell biology and molecular biology for providing innovative and cost-effective microelectronic biological analysis systems and applications for life science industry and clinical diagnostics
- ACEA’s first product was marketed under the brand name of RT-CES system in 2004
- In November of 2007 Roche and ACEA Biosciences entered into an exclusive agreement for the development, supply and distribution for ACEA Bioscience’s real-time cell assay technology. Under the terms of the agreement, RAS will exclusively market systems for real-time cell analysis, based on ACEA Bioscience’s impedance-based technology
- The first joint Roche/ACEA product is marketed under the brand name of xCELLigence
xCELLigence RTCA SP System

Computer and Software

Analyzer

E-Plate

Plate Reader (RTCA SP) (in CO2 incubator)

Gold Microelectrode Covers 80% of Well Area
Derivation of Cell Index

A dimensionless parameter termed Cell Index (CI) is derived as a relative change in measured electrical impedance to represent cell status.

Several features of the CI are summarized:

1. When cells are not present or are not well-adhered on the electrodes, then the CI is zero.

2. Under the same physiological conditions when more cells are attached on the electrodes, then the CI values are larger. Thus, CI is a quantitative measure of cell number present in a well.

3. Additionally, change in a cell status, such as cell morphology, cell adhesion or cell viability will lead to a change in CI.
Advantages of xCELLigence System for Cell-Based Assays and Drug Discovery Applications

- Label free, no reporters
- Non-invasive measurement
- Real-time Monitoring
  - Short-term (milliseconds)
  - Long-term (days and weeks)
- Continuous QC
Cell-based Assays:

Traditional Methods

Seed cells
Treatment: e.g.: drug compound
Initiate Experiment

Black Box
1-24 Hours

Traditional methods: Labeling (e.g. optical) & End-point Measurement

Data Analysis
Cell-based Assays:

Traditional Methods vs xCELLigence System

Seed cells

Treatment: e.g.: drug compound

Traditional methods:
Labeling (e.g. optical)
& End-point Measurement

Initiate Experiment

18-24 Hours

24-48 Hours

1-24 Hours

Data Analysis

Continuous QC

xCELLigence System:

• Label-free: Electronics-based detection
• Real-time: Continuous measurement, data analysis and display
• Therefore, both short term and long term compound effects can be captured
Applications Developed on the xCELLigence System

- Cell Proliferation
- Cell Quality
- Compound-mediated Cytotoxicity
- Cell-mediated Cytotoxicity
- Cell Adhesion and Spreading
- Functional Monitoring of Receptor Tyrosine Kinase Signaling
- Functional Monitoring of GPCR Signaling
- IgE Receptor Function
- Cell Invasion and Migration
- Barrier Function
- Viral Cytopathogenicity
Applications Developed on the xCELLigence System

- Cell Proliferation
- Cell Quality
- Compound-mediated Cytotoxicity
- **Cell Response Profiling**
- Cell-mediated Cytotoxicity
- Cell Adhesion and Spreading
- Functional Monitoring of Receptor Tyrosine Kinase Signaling
- **Functional Monitoring of GPCR Signaling**
- IgE Receptor Function
- Cell Invasion and Migration
- Barrier Function
- Viral Cytopathogenecity
Time-Dependent Cell Response Profiling
The Road to Cellular Cytotoxicity Takes Many Twists and Turns

Proteasome Inhibitor

N-Glycosylation Inhibitor

Anti-Mitotic

DNA Damaging
Are Impedance-Based Cell Response Profiles Predictive of Biological Mechanism?
TCRP Approach

Seed 4000 A549 Cancer Cells in 96 well E-Plates

Treat with Compounds at a final concentration
Of 20 μM

Spectrum Compound Library from MS Discovery
(Collection of FDA approved drugs, nature compounds
Experimental compounds, insecticides and herbicides)

Monitor the cellular response for 48 hours

Compare cytological profiles
Hit Selection and Clustering Analysis

TCRP from Screen

Short-term response (within 1 hour) → Hit criteria (25% of control)

Long Term Response (1-48 hours) → Hit criteria (25% and 40%)
TCRP with Known Mechanisms

Short-Term Response

- **Anti-Histamine**
  - Ctrl
  - Azelastine

- **Serotonin Receptor Antagonist**
  - Ctrl
  - Methiothepin

- **L-Type Voltage-gated Ca Channel Inhibitor**
  - Ctrl
  - Amlodipine

Long-Term Response

- **Anti-mitotics**
  - Ctrl
  - Colchicine

- **DNA Damaging**
  - Ctrl
  - Etoposide

- **Protein-Synthesis**
  - Ctrl
  - Emetine

- **Nuclear Hormone**
  - Ctrl
  - Hydrocortisone

- **HDAC Inhibitors**
  - Ctrl
  - Trichostatin A
TCRP with Known Mechanisms

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TCRP of Anti-mitotic Compounds
Characterization of Impedance-Based Anti-mitotic Profile

Mitotic Index

Ctrl 6 h (Phase I) 14 h (Phase II)

24 h (Phase III) 48 h (Phase IV)

Untreated 12.5 nM Paclitaxel

Mitotic Index (%) vs Time (h)

Normalized CI vs Time (h)
Validation of Mitotic Arrest Profile

Eg5 Small Molecule Inhibitor

S-Trityl-L-Cysteine

DMSO

anti-tubulin Ab

anti-PH3
Compounds with Anti-mitotic Profile from the Spectrum Collection

- Colchicine
- Paclitaxel

Benzimidazole

Podophyllotoxins

Curcumin
- Estradiol
- Noscapine HCL
- Griseofulvin
- Rotenone
- Chelidonine

SAPPANONES
Systematic Analysis of Impedance-Based Cell Response Profiles
Curve Classification Algorithm and Display
TCRP COX-2 Inhibitors

COX-2 Inhibitors

Valdecoxib

Rofecoxib

Celecoxib

Deracoxib

Valdecoxib

Rofecoxib

Deracoxib
TCRP COX-2 Inhibitors

COX-2 Inhibitors

Valdecoxib

Rofecoxib

Celecoxib

Deracoxib

Normalized CI vs Time (hours) for:
- Valdecoxib
- Rofecoxib
- Celecoxib
- Deracoxib
Validation of Celecoxib as Modulator of Intracellular Calcium Levels

![Graph showing the effect of BAPTA AM and 50 uM Celecoxib on normalized cell index over time. The graph includes curves for 10 uM BAPTA AM, DMSO, and Untreated conditions.]
Validation of Celecoxib as Modulator of Intracellular Calcium Levels

![Graph showing the effect of BAPTA AM and Celecoxib on intracellular calcium levels.](Image)

The graph illustrates the normalized calcium levels over time for different treatments: 10 uM BAPTA AM, DMSO, and Untreated. The x-axis represents time in hours, ranging from 26.7 to 27.3, while the y-axis represents normalized calcium levels.

Thapsigargin concentrations used were 50 uM, 25 uM, 12.5 uM, and 6.25 uM, with corresponding mean fluorescence (Arbitrary Units) shown in the bar graph on the right side of the slide.
Advantages of Short Term and Long Term Monitoring of Cellular Response Profiles

Monastrol

HeLa Cells

Normalized CI

Time (hours)
Advantages of Short Term and Long Term Monitoring of Cellular Response Profiles

Monastrol

HeLa Cells

Normalized CI vs Time (hours)
Advantages of Short Term and Long Term Monitoring of Cellular Response Profiles

Monastrol

HeLa Cells

Normalized CI

Time (hours)
Advantages of Short Term and Long Term Monitoring of Cellular Response Profiles

Monastrol

HeLa Cells

Normalized CI

Time (hours)

Long Term

Ctrl

Monastrol

Phospho-Histone H3
Advantages of Short Term and Long Term Monitoring of Cellular Response Profiles
Advantages of Short Term and Long Term Monitoring of Cellular Response Profiles

Monastrol

HeLa Cells

Short Term

Voltage-Gated Calcium Level Modulation

Long Term

Phospho-Histone H3

Ctrl

Monastrol

nifedipine
Functional Monitoring of GPCR Signaling
GPCR Activation Leads to Modulation of the Actin Cytoskeleton
Dynamic Monitoring of GPCR-mediated Morphological Dynamics Using the xCELLigence RTCA System

![Graph showing normalized CI over time for Histamine and Vasopressin](image)

- **Histamine**
  - CTR: 1.2
  - Stimulated: 2.0 (peaks at 10h)

- **Vasopressin**
  - CTR: 1.4
  - Stimulated: 1.8 (peaks at 11h)

**Immunofluorescence Images**

- **Unstimulated**
  - Nuclei (green), Phalloidin (red), anti-Paxillin (white)

- **+ Histamine**
  - Increased cell spreading

- **+ Vasopressin**
  - Increased cell spreading

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Functional Monitoring of Gq Coupled Receptors on the xCELLigence RT-CA System

**Histamine H1 Receptor**

- Normalized CI over time for various concentrations of histamine:
  - His 30 uM
  - His 30 nM
  - His 30 pM
  - CTR

**RT-CA Assay**

- Normalized CI vs. [Histamine] Log M
- EC\textsubscript{50} = 1.7 nM

**3H-IP\textsubscript{3} Assay**

- [\textsuperscript{3}H]IP\textsubscript{3} bound (cpm)
- EC\textsubscript{50} = 19 nM
Functional Monitoring of Gₛ Coupled Receptors on the xCELLigence RT-CA System

Dopamine1 Receptor

RT-CA Assay

cAMP Assay

- **EC₅₀** = 1.7 nM
- **EC₅₀** = 1.9 nM
Functional Monitoring of $G_i$ Coupled Receptors on the xCELLigence RT-CA System

5-HT1A Receptor

RT-CA Assay

cAMP Assay

- EC$_{50} = 19$ nM
- EC$_{50} = 6$ nM
Dynamic Monitoring of Endogenous Receptors Using the xCELLigence System

- **Histamine Receptor (Gq) in HeLa Cells**
  - EC50 = 146 nM

- **Calcitonin Receptor (Gs) in CHO Cells**
  - EC50 = 385 pM

- **Opioid Receptor (Gi) in NIE115 Cells**
  - EC50 = 2 nM
Dynamic Monitoring of Receptors in Disease-Relevant Cell Types

Cor.AT Cells from Axiogenesis

Mouse ES Cell-Derived Cardiomyocytes
100% Pure Population

β2 Adrenergic Receptor Activation in Cardiomyocyte-Differentiated Cor.AT Cells

EC₅₀ = 3.1 nM
Identification of Histamine H1 Receptor Inverse Agonist using the xCELLigence RT-CA System

- **Histamine 100 nM**
- **Loratidine 100 uM**

**Graphs:**
1. **Top graph:**
   - **Time (h):** 6, 8, 10, 12, 14, 16, 18
   - **Normalized CI:** 0.8, 1, 1.2, 1.4, 1.6, 1.8, 2

2. **Bottom graph:**
   - **Time (h):** 10, 12, 14, 16, 18, 20
   - **Normalized CI:** 0.7, 0.8, 0.9, 1, 1.1, 1.2, 1.3, 1.4, 1.5
List of Receptors Functionally Monitored by the xCELLigence System

- GPCR
- RTK
- FcR (IgE and IgG)
- TCR
- Death Receptors (FasR, TNFR)
- Integrins
- Toll Receptors
- Nuclear Hormone Receptors
Summary

- Impedance-based monitoring of cellular status using the xCELLigence platform allows for monitoring of both short term and long term responses.

- The ability to monitor short and long term responses within the same experiment provides cytological profiles which can be predictive of mechanism of action.

- The non-invasive nature of impedance readout provides the advantage of working with primary cells or disease relevant cells both for long term cytotoxicity studies and short term receptor responses.