

Continuous OCR Monitoring Detects Mitochondrial Cardiotoxicity Ahead of Visible Cytotoxicity in iPSC-Derived Cardiomyocytes

A functional metabolic readout for preclinical cardiac safety assessment

In collaboration with **Cellomatics Biosciences**

The Metabolic Dimension of Cardiac Safety

Drug-induced cardiotoxicity (DICT) is one of the leading causes of drug attrition and post-market withdrawal. Current preclinical safety guidelines (ICH S7B, E14) focus primarily on ion channel electrophysiology, particularly inhibition of the hERG potassium channel. While essential, these assays do not capture the metabolic dimension of cardiac toxicity.

Mitochondrial dysfunction is an established mechanism of DICT, particularly for kinase inhibitors, anthracyclines, and other oncology agents. Drug-induced impairment of oxidative phosphorylation reduces ATP production in cardiomyocytes, driving contractile dysfunction and cell death without necessarily triggering ion channel-based alerts.

Resipher (Lucid Scientific) monitors oxygen consumption rate (OCR) continuously over days to weeks in standard 96-well plates, without assay media swaps, fluorescent dyes, or plate transfers. Applied to iPSC-derived cardiomyocytes, it provides a sensitive, non-invasive window into mitochondrial health in $\text{fmol}/\text{mm}^2/\text{s}$, throughout a compound exposure and recovery period.

KEY HIGHLIGHTS

CoCl_2 exposure detected by OCR, with no cytotoxicity visible at the same timepoint

Bioenergetic profiling identified preferential ATP-linked respiration loss consistent with Complex IV inhibition

Dose-dependent, persistent metabolic impairment quantified continuously over 6 days post-exposure

Full safety dataset from a single 14-day experiment with no assay media swap

Experimental Model

IPSC-DERIVED VENTRICULAR CARDIOMYOCYTES AND RESIPHER SETUP

Human iPSC-derived ventricular cardiomyocytes (iPSC-vCMs; AXOL, ax2508) were thawed and cell viability assessed by trypan blue exclusion prior to seeding in fibronectin-coated 96-well plates. 56,000 viable cells were seeded per well. All experiments were conducted independently by Cellomatics Biosciences (Nottingham, UK) under contract. Continuous OCR monitoring used the Resipher NS96 sensing lid from Day 1 of culture.

IPSC-VCM MATURATION AND FUNCTIONAL VALIDATION

Cells underwent a 7-day maturation protocol with stepwise introduction of MyoMax Maturation medium (25% on Day 5, 50% on Day 7). OCR rose from 15 fmol/mm²/s at seeding to over 100 fmol/mm²/s by Day 7, demonstrating progressive engagement of oxidative phosphorylation. Brightfield imaging confirmed rod-shaped morphology by Day 7, consistent with ventricular cardiomyocyte maturation. At the point of CoCl₂ application on Day 8, OCR had reached 75–90 fmol/mm²/s, providing a metabolically active baseline for compound exposure.

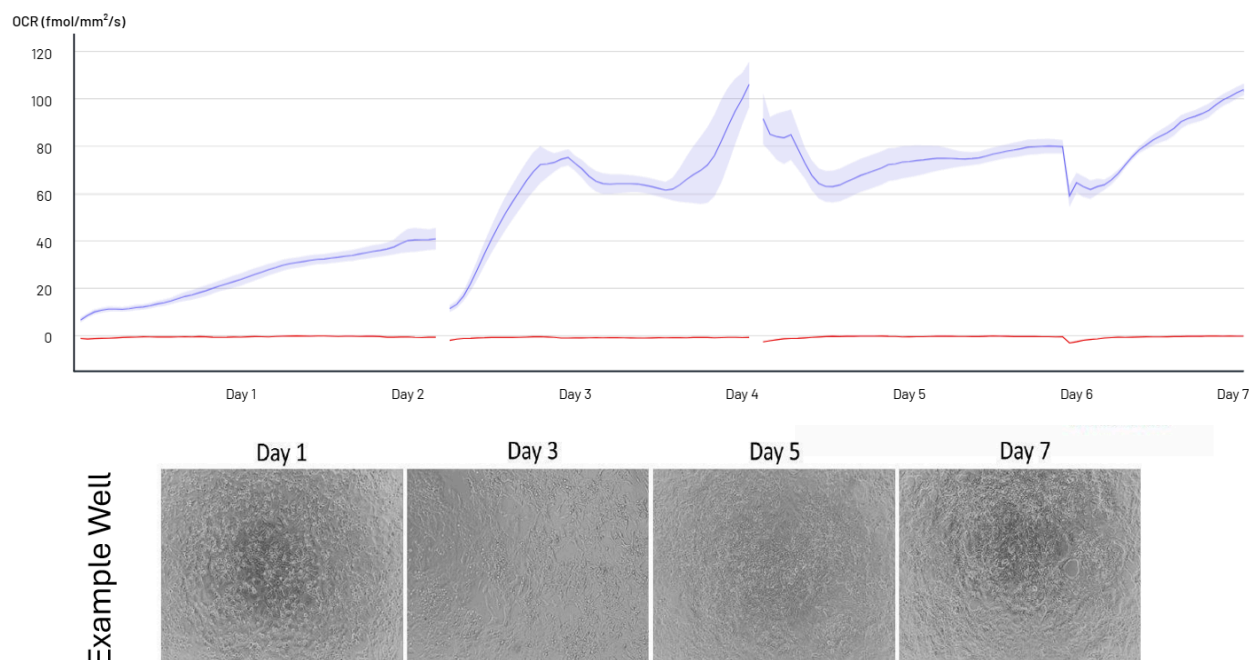


Figure 1. iPSC-vCM maturation over Days 1-7. Top: Continuous OCR (fmol/mm²/s) for iPSC-vCM (blue) vs No Cells (red); OCR rises from ~15 to >100 fmol/mm²/s by Day 3, stabilizing at ~75–90 fmol/mm²/s by Day 6, consistent with progressive engagement of oxidative phosphorylation. Bottom: Brightfield images (vehicle control, 56,000 cells/well); rod-shaped morphology evident by Day 7. ×10 magnification, Optika inverted microscope. Courtesy of Cellomatic Biosciences.

CHEMICAL HYPOXIA AS A MODEL CARDIOTOXICANT

Cobalt chloride (CoCl₂) was used as a model cardiotoxicant and chemical hypoxia mimetic. CoCl₂ stabilizes HIF-1α and inhibits Complex IV (cytochrome c oxidase), directly reducing mitochondrial respiration in a manner relevant to ischemia and drug-induced cardiotoxicity. On Day 8, CoCl₂ was applied for 24 hours then removed: 12.5–100 μM (vehicle: 0.1% dH₂O).

A bioenergetic profiling (BEP) experiment then characterized the mechanism of impairment. ETC modifier compounds were applied on Day 12: oligomycin (1 μM), FCCP (2 μM), and rotenone/antimycin A (0.5 μM); vehicle: (0.2% DMSO). As Resipher monitors without media injection, BEP was conducted using a parallel well design: individual wells each received a single ETC modifier, with mean OCR responses compared across groups to derive bioenergetic parameters. No formal statistical testing was applied; all data represent mean ± SEM from n = 3 independent wells per concentration.

Subclinical Window: Metabolic Impairment Persists After Washout

A single 24-hour CoCl₂ exposure produced lasting, dose-dependent mitochondrial impairment in iPSC-vCMs, detectable for at least six days after compound removal. Mean OCR over Days 9–12 fell to 0.89-fold (12.5 μM), 0.78-fold (25 μM), and 0.71-fold (50 μM) relative to the pre-treatment basal reference (vehicle: 1.2-fold, reflecting continued maturation). Brightfield imaging showed no visible cytotoxicity at any of these concentrations.

CONTINUOUS OCR NORMALIZED TO PRE-TREATMENT BASAL (0-50 mM CoCl_2)

Figure 2 shows mean relative OCR (fold change from pre-treatment baseline) across all four CoCl_2 concentrations over 6 days of treatment, with bar charts at four post-exposure timepoints: sustained, dose-ordered suppression is evident at all timepoints.

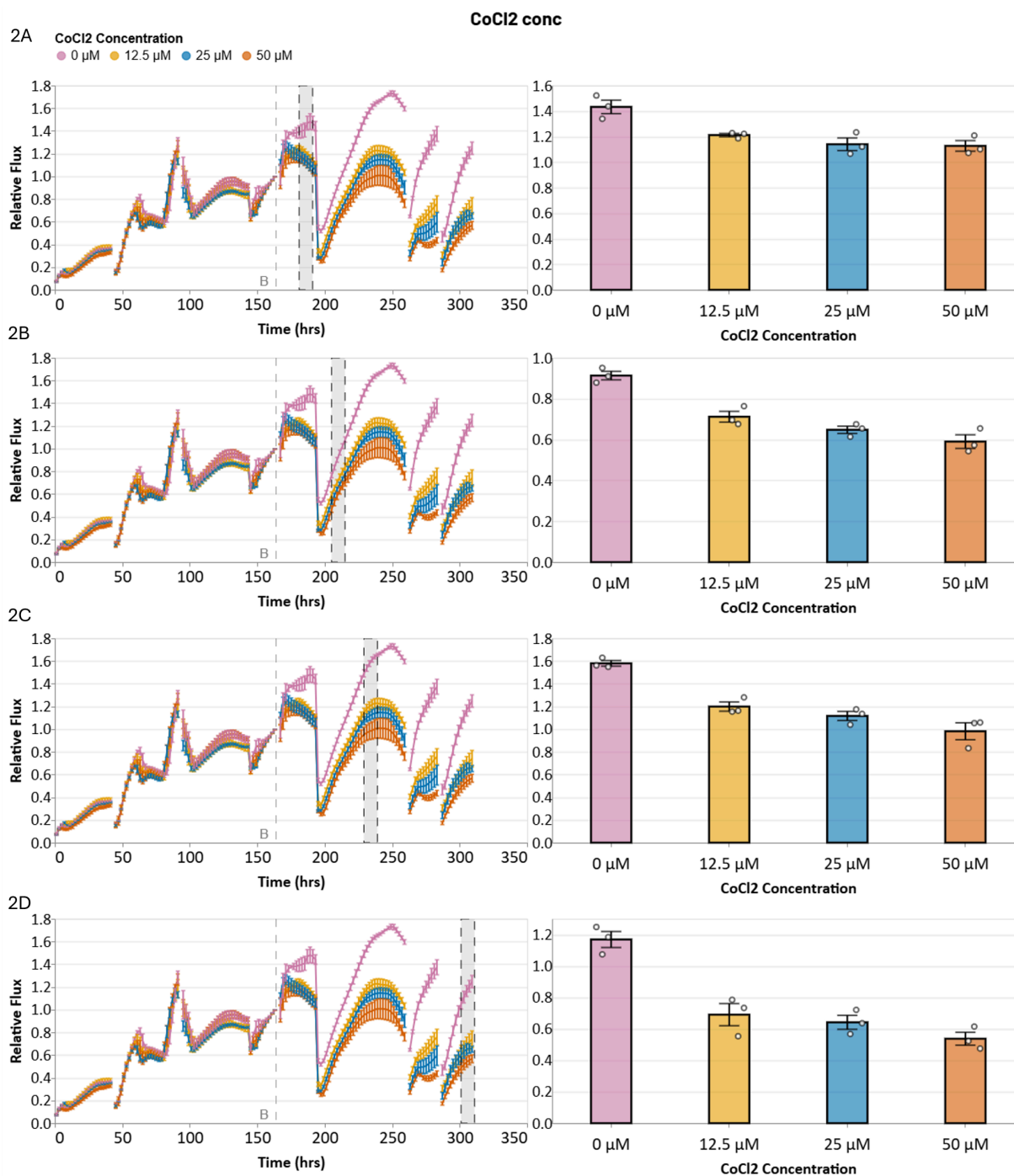
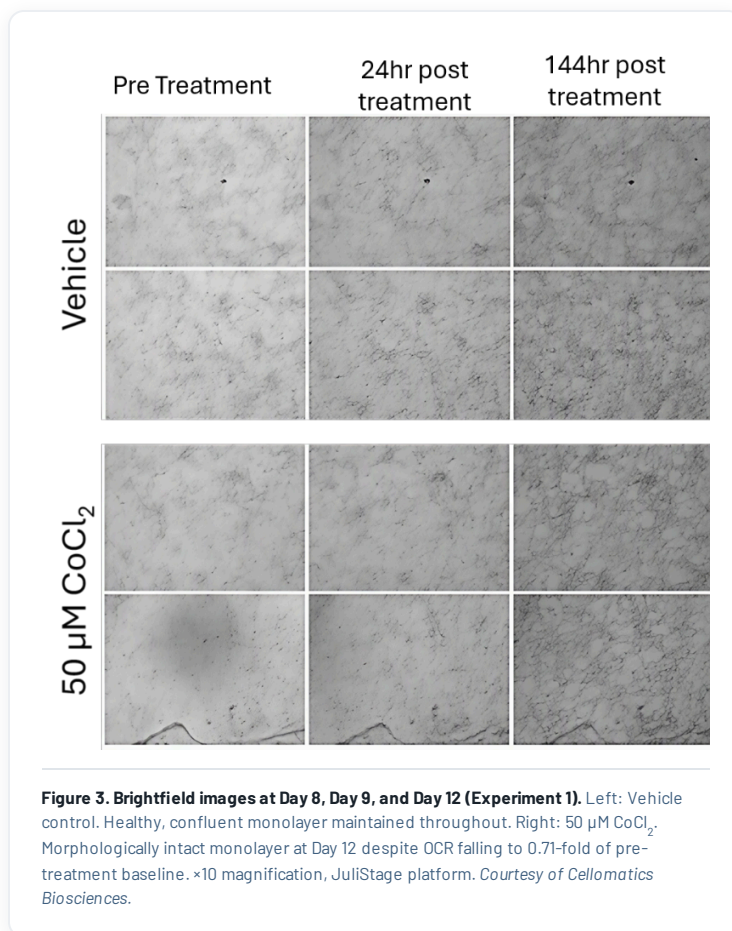


Figure 2. Continuous Resipher OCR monitoring (Experiment 3, 0-50 μM CoCl_2). Left: mean relative OCR (fold change from pre-treatment baseline, $t = 160$ h) over 6 days; grey shading indicates timepoints shown in bar charts. Dashed line: CoCl_2 addition (160h). Right: mean $\pm$ SEM at 2A, 24hrs post application of CoCl_2 (drug still in the cells), 48 hours post application (2B), 72 hours post application (2C) and 144hrs (6 days)(2D) post application of CoCl_2 . Open circles = individual wells. $n=3/\text{concentration}$.

BRIGHTFIELD MORPHOLOGY: NO VISIBLE CYTOTOXICITY AT SUBCLINICAL DOSES

At 50 μM CoCl_2 , where OCR fell to 0.71-fold of the pre-treatment baseline, cells remained morphologically intact through Day 12.



THE SAFETY SCREENING IMPLICATION

At 12.5–50 μM CoCl_2 , OCR fell to 0.71–0.89-fold of pre-treatment baseline (Figure 2) with intact cell morphology at Day 12 (Figure 3). In a standard cardiac safety screen relying on endpoint viability or morphology alone, all three concentrations would be classified as inactive at Day 9, and the compound would advance. Continuous OCR monitoring identifies this subclinical metabolic liability, the type most likely to manifest as delayed cardiotoxicity in vivo. The timepoint-by-timepoint progression underlying this finding is examined in detail below.

Dose-Dependent OCR Suppression Across a Concentration Range

Four representative timepoints were selected: 24 hr post dosing, 48 hours post dosing (24hr of CoCl_2 removed), 72hr post dosing, and experimental endpoint 144 hr post dosing. All OCR values are expressed as fold change relative to the pre-treatment basal reference window ($t=166\text{h}$) in Figure 4A.

At 24 hours post-dosing, CoCl_2 -treated cells showed modest OCR suppression relative to vehicle: 12.5 μM fell to 0.91-fold of the pre-treatment baseline (± 0.019 SEM), 25 μM to 0.85-fold (± 0.034), and 50 μM to 0.85-fold (± 0.037), compared to 1.21-fold in vehicle-treated cells continuing to mature (4A). Taken in isolation, this early readout might suggest mild, dose-independent suppression, an inconclusive result in a conventional single-endpoint assay. But continuous monitoring told a different story.

By 48 hours post application, while vehicle OCR peaked at 1.81-fold (± 0.007), the CoCl_2 groups had fallen further behind: 12.5 μM reached 1.27-fold (± 0.041), 25 μM 1.18-fold (± 0.055), and 50 μM just 1.04-fold (± 0.088), no longer tracking the maturation-driven rise seen in controls at all.

By the experimental endpoint, six days after compound removal, suppression had deepened further still: 12.5 μM had partially recovered to 1.02-fold (± 0.081), while 25 μM remained at 0.79-fold (± 0.094) and 50 μM at 0.63-fold (± 0.038), well below the pre-treatment baseline, with no cytotoxicity visible in brightfield at any concentration.

During the CoCl_2 dosing window, vehicle control OCR continued to rise above the pre-treatment baseline, reaching 1.51-fold at 24 hours and 1.67-fold at 72 hours post-dosing. This reflects ongoing cardiomyocyte maturation independent of treatment. When baseline-normalized, CoCl_2 -treated wells at these timepoints therefore appear elevated above 1.0, despite being substantially suppressed relative to untreated cells. Normalizing to the concurrent vehicle control removes this confound and reveals the true extent of metabolic impairment.

When expressed as a fraction of vehicle OCR, suppression was present and dose-ordered at every timepoint. At 24 hours post-dosing, 12.5 μM CoCl_2 reduced OCR to 0.85-fold of vehicle (± 0.010 SEM), 25 μM to 0.79-fold (± 0.035), and 50 μM to 0.79-fold (± 0.031). By 72 hours, with vehicle OCR at its peak, the separation between groups widened: 12.5 μM fell to 0.76-fold (± 0.026), 25 μM to 0.71-fold (± 0.027), and 50 μM to 0.62-fold (± 0.048) of vehicle. At the experimental endpoint, 144 hours post-dosing, suppression was deepest across all doses: 12.5 μM at 0.60-fold (± 0.062), 25 μM at 0.55-fold (± 0.041), and 50 μM at 0.46-fold (± 0.037) of vehicle. Unlike the baseline-normalized data, vehicle normalization shows a consistent, dose-ordered signal at all four timepoints, with no ambiguity at the timepoints where vehicle OCR is elevated. LucidLab's built-in normalization options allow the analysis method to be matched to experimental design, a practical advantage when vehicle OCR is not stable across the monitoring window.

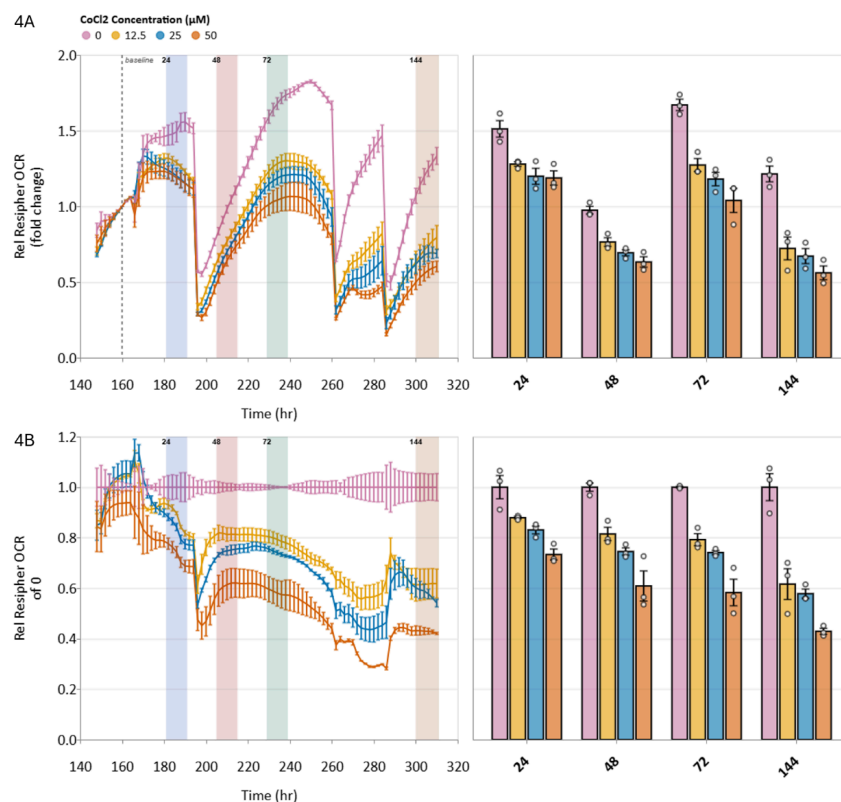
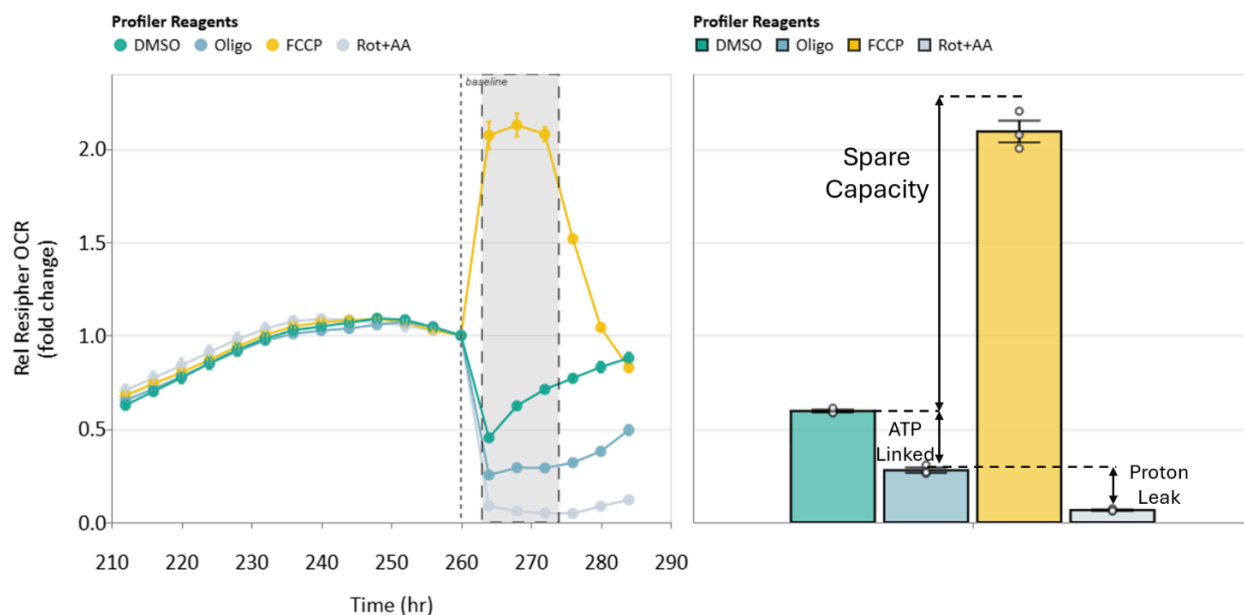


Figure 4. Continuous Resipher OCR monitoring. Left: mean relative flux (fold change to pre-treatment baseline, $t=160\text{h}$); shaded bands = SEM. Right: mean $\pm$ SEM at 24 hr post dosing, 48hr post dosing, 72 hr post dosing and 144 hours post dosing ($n=3/\text{concentration}$).

The full liability was not apparent at any single timepoint; it emerged through the progressive OCR trace. This is also highlighted when dose-response was normalized to a vehicle control.

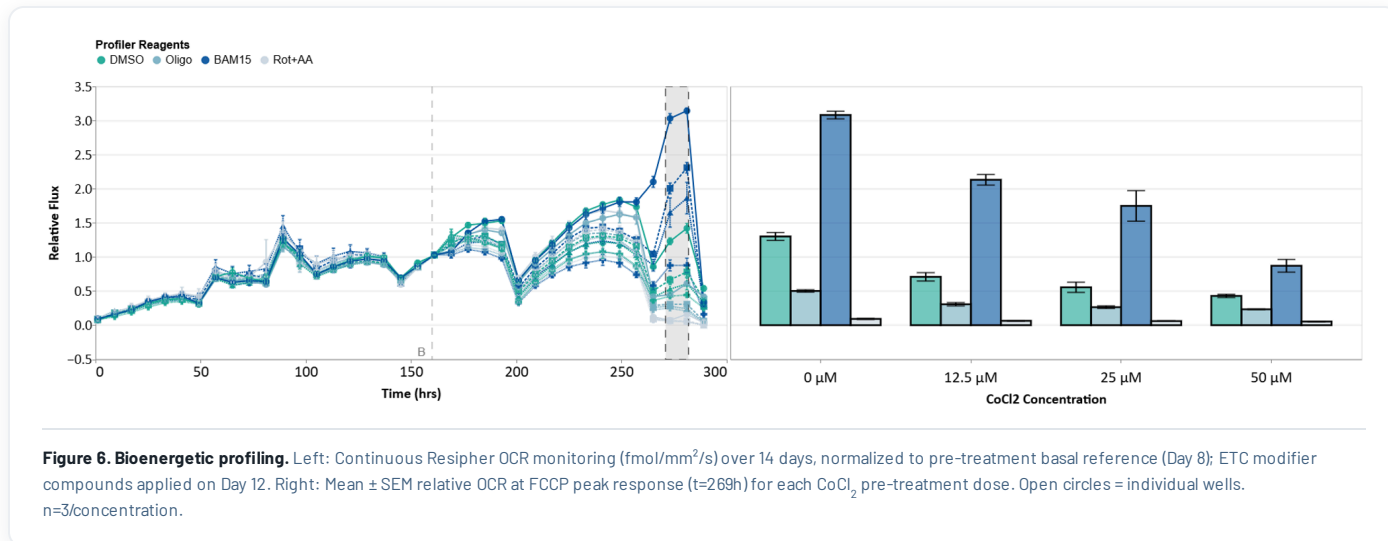
Mechanistic Characterization: What Kind of Toxicity?

Bioenergetic profiling (BEP) was performed on iPSC-vCMs pre-treated with sub-cytotoxic CoCl_2 doses (12.5, 25, 50 μM). ETC modifier compounds were applied on Day 12 and OCR responses used to derive key bioenergetic parameters.



BIOENERGETIC PARAMETER	VEHICLE	12.5 μM	25 μM	50 μM
Basal OCR (fmol/ mm^2/s)	1.237	0.673	0.534	0.429
Non-mitochondrial OCR	0.073	0.064	0.064	0.057
ATP-linked respiration	0.781	0.366	0.259	0.184
Proton leak	0.383	0.243	0.212	0.187
Maximal respiration (FCCP)	2.963	1.955	1.601	0.814
Spare respiratory capacity	1.798	1.347	1.131	0.442
Coupling efficiency	67%	60%	55%	50%

Table 2. Bioenergetic profiling parameters in iPSC-vCMs pre-treated with CoCl_2 (24h, Day 8) vs vehicle. ETC modifiers applied for 24h (vehicle: 0.2% DMSO). All OCR values in fmol/ mm^2/s .



ATP-linked respiration fell to 0.47-fold (12.5 μM CoCl_2), 0.33-fold (25 μM), and 0.24-fold (50 μM) relative to vehicle, a disproportionately large loss compared with the corresponding falls in maximal respiration (0.66-, 0.54-, and 0.27-fold respectively). This pattern, combined with a progressive decline in coupling efficiency from 67% to 50% and a dose-dependent rise in the proton leak fraction, is consistent with primary Complex IV inhibition driving secondary mitochondrial uncoupling. CoCl_2 also stabilizes HIF-1 α , which may contribute a transcriptional component to OCR suppression at higher concentrations; the BEP pattern of disproportionate ATP-linked loss is most consistent with direct ETC inhibition at the doses tested but a HIF-1 α -mediated contribution cannot be excluded. Spare respiratory capacity collapsed to 0.25-fold of vehicle at 50 μM . In cardiomyocytes with high and fluctuating ATP demand, loss of this reserve represents a functional deficit that would not register in a viability or morphology screen but has direct implications for contractile function under physiological stress.

Conclusions

Continuous OCR monitoring with Resipher detected functional mitochondrial cardiotoxicity in iPSC-CMs at concentrations where cells appeared non-toxic under brightfield imaging for days after compound removal. The OCR response was quantitative, persistent, and mechanistically characterizable from a single 14-day experiment with no assay media swap or reagent injections.

The metabolic liability detected here at sub-cytotoxic concentrations mirrors the pattern seen with clinical cardiotoxins including anthracyclines and tyrosine kinase inhibitors, where mitochondrial impairment precedes overt cell death and is missed by standard viability endpoints.

Resipher adds a metabolic safety dimension that electrophysiology-based assays do not provide. Used alongside existing cardiac safety screens, it enables earlier, more-complete characterization of compound liability, reducing the risk of metabolic cardiotoxicity advancing through late-stage development.

See Resipher in action

Request a virtual demo and talk through your cardiac safety workflow with our applications team.

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Supplementary Information

BIOENERGETIC PARAMETER	CALCULATION
Basal OCR	Measured directly. Steady-state OCR immediately before ETC modifier addition
Non-mitochondrial OCR	OCR remaining after rotenone + antimycin A (Complex I/III fully blocked)
ATP-linked respiration	Basal OCR – OCR after oligomycin
Proton leak	OCR after oligomycin – Non-mitochondrial OCR
Maximal respiration (FCCP)	OCR after FCCP – Non-mitochondrial OCR
Spare respiratory capacity	Maximal respiration – Basal OCR
Coupling efficiency	$\text{ATP-linked respiration} \div (\text{Basal OCR} - \text{Non-mitochondrial OCR}) \times 100\%$

Table 3. Derivation of bioenergetic parameters (Table 2) from raw OCR measurements taken at basal, and following addition of oligomycin, FCCP, and rotenone/antimycin A. Coupling efficiency is expressed as a percentage of mitochondrial (non-mitochondrial-subtracted) basal OCR.

REFERENCES

- 1) ICH S7B (2005) and E14 (2005) guidelines on the nonclinical and clinical evaluation of QT/QTc interval prolongation.
- 2) Dykens, J. A., & Will, Y. (2007). The significance of mitochondrial toxicity testing in drug development. *Drug Discovery Today*, 12(17-18), 777-785.
- 3) Experimental data generated by Cellomatic Biosciences (Nottingham, UK) under contract for Lucid Scientific. Study performed by Klaudia Rzepecka, PhD (Bioassay Scientist II), reviewed by Alex Timmis, PhD (Bioassay Study Manager), 18 June 2026. Data ownership: Lucid Scientific.

lucidsci.com | info@lucidsci.com