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**HeCaToS**

Collaborative project

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Modelling toxic response in case studies for predictive human safety assessment

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**Deliverable Report D8.8:**

**xCELLigence data set describing the impact of drug treatment on the  
electrophysiology of a 3D heart model**

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Work package 8

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<b>PP</b>	Restricted to other programme participants (including the Commission Services)	
<b>RE</b>	Restricted to a group specified by the consortium (including the Commission Services)	
<b>CO</b>	Confidential, only for members of the consortium (including the Commission Services)	x

## Contributions to deliverable - Internal review procedure

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## PUBLISHABLE SUMMARY

Data describing the impact of drug treatment on contractility of a 2D cardiac model.

## OBJECTIVES

The generation of data sets describing the impact of drug treatment on the electrophysiology of a cardiac *in vitro* model.

## INTRODUCTION

Lead compounds are frequently terminated at late stages of drug development due to cardiac safety concerns. Such concerns can relate to a variety of adverse drug effects on the heart but a key consideration is altered cardiomyocyte ion channel activity resulting in a cardiac arrhythmia.

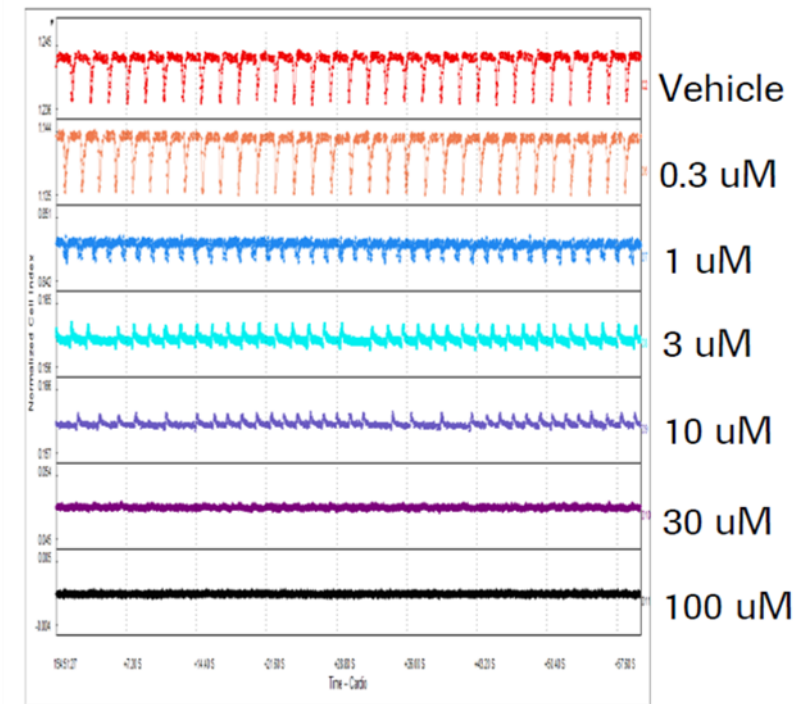
The xCELLigence RTCA Cardio system utilises a non-invasive impedance readout for real time monitoring of cardiomyocyte beating activity and can therefore provide relevant mechanistic toxicity data with two key metrics being:

- i. The lowest concentration displaying 20% irregular beats (IB20) and
- ii. The lowest concentration inducing an alteration in beat rate of  $\geq 20\%$  compared with a time matched vehicle control (BR20). These measurements are only applicable to cells in 2D as contact is required with place sensors.

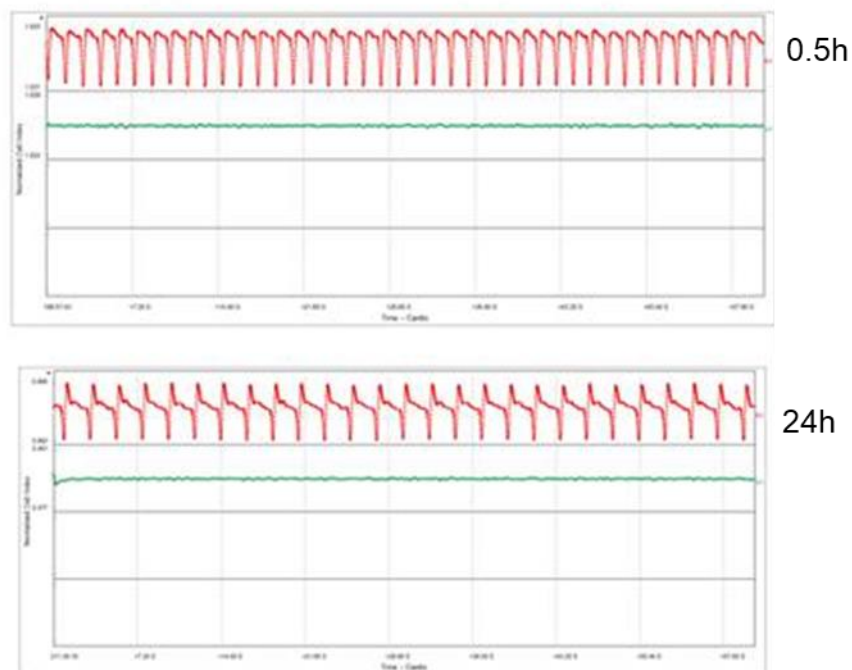
In conjunction with Task 8.2 the capacity has also been developed to measure mitochondrial function AND contractility in sequence on the same cell type by establishing a method to measure mitochondrial function on xCELLigence plates (see Deliverable Report D8.2). This, for the first time, links 'work' conducted by the cardiac model to the metabolic activity required to drive that work on a microplate format.

## RESULTS

The impact of Doxorubicin and Amiodarone on cardiomyocyte function was tested and representative data is presented in Fig. 1 and 2 illustrating an IB20 value of  $1\mu\text{M}$  and a BR20 value of  $10\mu\text{M}$ . These data have been submitted to WP9.



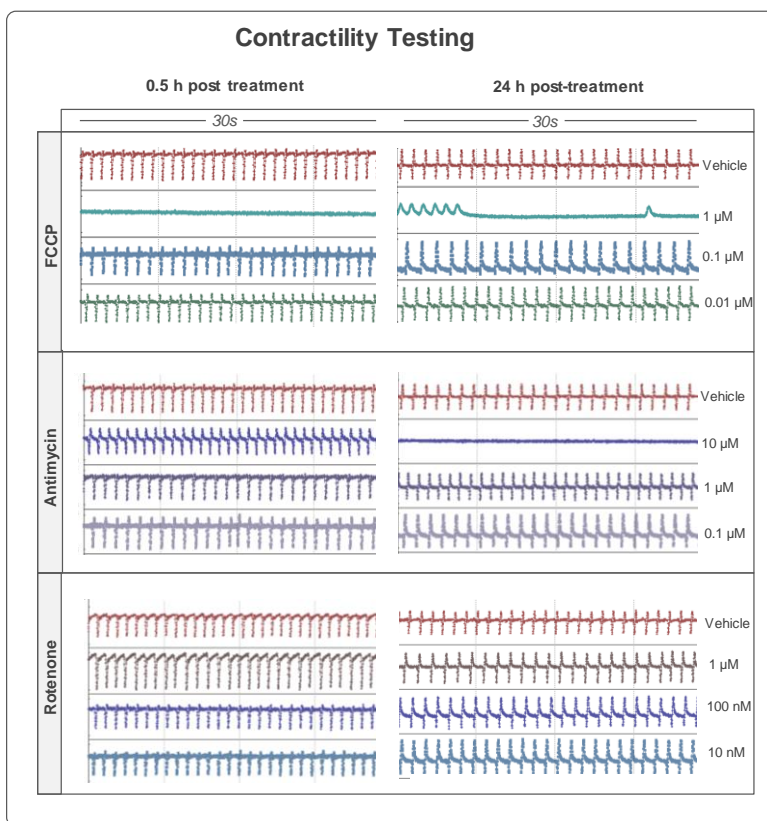
**Fig 1:** Beating profile at 48 h of treatment with Doxorubicin



**Fig 2:** Beating profile at 48 h of treatment with Amiodarone

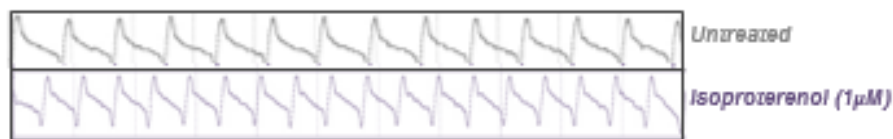
As outlined above in T8.2, further advancements in demonstrating sequential analysis of cardiomyocyte mitochondrial function, metabolism and beating through combined measurements on xCELLigence e-plates was described. Data presented includes the corresponding beating profiles for the same compound treatments including classical mitochondrial compounds to assess assay performance (Fig. 3), as well as treatments of known cardio-active drugs which alter beat rate such as Isoproterenol,

Nifedipine and E-4031, to assess specific cardiomyocyte responses (Fig. 3 & 4). Cardiomyocyte beating continues in the presence of the three classical compounds and is maintained for over 24h across most concentrations in Fig 3, as outlined above, this suggests that the measured increased glycolytic flux supplies sufficient ATP to facilitate cardiomyocyte beating despite complete impairment of OxPhos, seen as decrease in measured O<sub>2</sub> consumption (Fig 3). Only at maximal concentration for FCCP (1μM) and Antimycin (10μM) after 24 h's is contractility seen to be impaired.



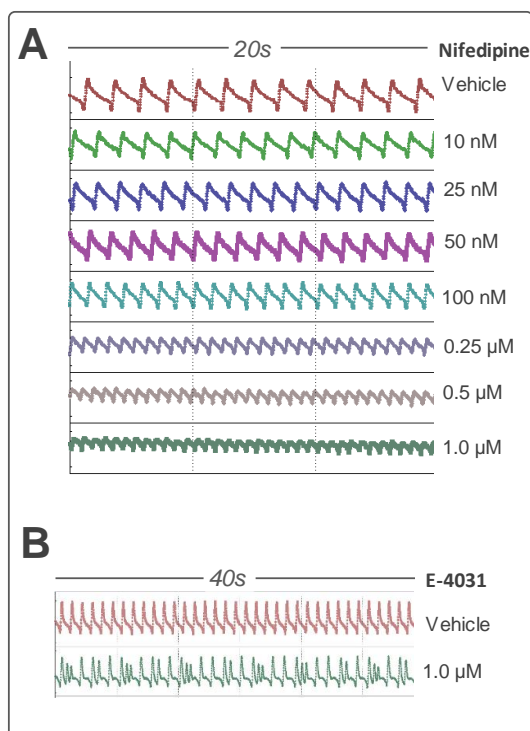
**Fig 3:** Beating profiles at 0.5h and 24 h of treatment with classical ETC compounds, FCCP, Antimycin and Rotenone, linked to mitochondrial data in Fig 6.

Treatment with the β-adrenoreceptor agonist isoproterenol causes an increase in cardiomyocyte beat rate, shown in Fig 3, corresponding impact on cardiomyocyte metabolism described above in T8.2.



**Fig 4:** Treatment of (β-adrenoreceptor agonist) isoproterenol causes an increase in cardiomyocyte beat rate (A), (data presented in D8.2 describes how this increased beating increases metabolic flux).

Treatment with L-type Ca<sup>2+</sup> channel antagonist nifedipine, decreases cardiomyocyte beating. Data shows the measured dose dependant effect of nifedipine treatment on cardiomyocyte beating Fig. 4A, and the effect the hERG channel inhibitor E-4031 on cardiomyocyte beating is shown in Fig. 4B (corresponding to metabolism data shown in D 8.2 whereby the perturbed beating observed here (measured at 30 min) cause significant reductions in O<sub>2</sub> consumption are also observed).



**Figure 4:** Illustrates the dose dependant decrease in cardiomyocyte beat rate caused by nifedipine treatment (A) linked to decreased O<sub>2</sub> consumption in D8.2, and the effect the hERG channel inhibitor E-4031 on both cardiomyocyte beating (B) which can be compared to effect on metabolism seen in D8.2.

This combined measurement of cardiomyocyte beating and cell metabolism is a significant advance and generates a more complete picture of cardiomyocyte response to drug treatment, helping describe the inter-relationships between beat rate and metabolism.

## DIFFICULTIES

While data has been generated describing the impact of drug treatment on the contractility of *an in vitro* cardiac model (above), due to specific inherent advantages, as the project progressed, the consortium chose to pursue the spheroid model exclusively. This meant that measuring such impedance-based outputs not broadly applicable, as contact is required between cell and sensor (2D model required).

The consortium also chose to pursue an innovative long-term repeat-dosing model which is not compatible with iPS derived cardiomyocytes in 2D and so the measurement parameter described here has not been broadly deployed across the cardiac drug list. It will however serve as an investigative tool to probe finding as they arise through data analysis where links between contractility and metabolism may be identified.

## REFERENCES

C. Carey, C. Bertinetti-Lapatki, A. Roth, J. Hynes. Assessing the Impact of Drug Treatment on Cardiomyocyte Function Through Combined Analysis of Beating, Metabolic Flux and Cellular Oxygenation. Society of Toxicology Annual Meeting, 201