Case study

In vitro diabetic cardiomyopathy model to evaluate ischemic sensitivity

Tools:
CardioExcyte 96

Dr. Yuanzhao (Nick) Cao,
featured by Nanion Technologies

Dr. Yuanzhao (Nick) Cao, University of Queensland. His work focuses on cardiovascular development, stem cell-based disease modeling and pharmaceutical drug discovery. Specifically, he concentrates on ischemic heart injury and diabetic cardiomyopathy in preclinical cell and animal models, aiming to develop new drugs to reduce hospitalizations and community health burdens.

Individuals with diabetes have a higher risk of developing ischemic heart disease, making it the leading cause of mortality among diabetics [1]. Limited preclinical models for co-morbidity of diabetes and ischemia are currently available to address this significant clinical concern.

Dr. Nick Cao from the University of Queensland aims to develop *in vitro* co-morbidity models to study ischemic sensitivity in preclinical models of diabetes. The researcher explains: "Individuals living with diabetes have frequent and higher magnitude glucose spikes. We therefore evaluated variable (vG) vs. constant (cG) glycemia in models of diabetic stress. This novel two-step diabetic cardiomyopathy (DCM) model mimics the postprandial glucose spikes measured using a monitor in patients to assess blood glucose changes throughout the day."

In the study shown here, human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) were exposed to control maturation media (MM) vs diabetic conditions. Constant glucose stress (cG) vs cyclic episodes of high and low glucose (vG), which mimic the glycemic stress of diabetic patients, were investigated. Compared to control MM conditions, both cG and vG cardiomyocytes showed greater lipotoxicity and cell stress characterized by increased intracellular uptake of lipid (Oil red O staining, ORO), as well as an increased expression of the heart failure bio-marker, brain natriuretic peptide (BNP). However, there was no significant difference in phenotypes between the two disease models.

Next, *in vitro* DCM models were investigated under physiological conditions, by analyzing cell contractility using the CardioExcyte 96. This platform allows recording of contractility and electrophysiology of cardiac cells [2,3]. DCM cells show impaired contractile performance; displaying decreased amplitude, beat rate, and compromised velocity. Interestingly, vG heart cells had greater defects in contractions compared to cG conditions, reflecting a key distinction between the two groups on the basis of cardiac physiology. In contrast, RNA-seq data demonstrate that while MM and diabetic models have significant differences, cG and vG

Nanion's CardioExcyte 96 platform
The CardioExcyte 96 is a compact, automated, hybrid technology, combining several functional readouts under physiological conditions. Recording from 96 wells at a time, CardioExcyte 96 captures both contractility and electrophysiology, with additional insight into cell viability.

> **"The CardioExcyte has played a pivotal role in advancing our comprehension of cardiac physiology and has become an indispensable tool in our research endeavors."**
>
> **Nathan J. Palpant**, Prof. Dr., University of Queensland

cells have similar gene expression changes. These include alterations in insulin resistance, fatty acid oxidation (FAO), glucose oxidation, and signaling pathways associated with DCM.

Figure 1 gives an overview of the *in vitro* DCM model to evaluate ischemic sensitivity. By exposing healthy hiPSC-CMs to increased glucose and neurohormonal mediators, novel glycemic variability (vG) and constant glucose models (cG) were developed (Figure 1 A). The disease models were further used to study exposure to ischemic stress using *in vitro* IRI modeling. As shown in Figure 1 B, diabetic cardiomyocytes (cG and vG) displayed increased lipotoxicity (i-ii) characterized by increased intracellular uptake of lipids and enhanced release of the DCM stress marker BNP (ii). Differential gene expression classified in clusters among MM, cG and vG conditions is shown in Figure 1 C. Key transcriptional hallmarks of the DCM model include insulin resistance, metabolic reprogramming and altered functionality.

Investigation of possible phenotypic changes revealed impaired contractile performance of the diabetic cardiomyocytes (i, ii), indicated by decreased contractile amplitude, beat rate, and compromised velocity (Figure 2).

"The CardioExcyte system has played a pivotal role in advancing our comprehension of cardiac physiology and has become an indispensable tool in our research endeavors." was one insight from the team at the University of Queensland.

Nick Cao continues: "Its ability to offer real-time, label-free measurements of contractility provides unparalleled insights

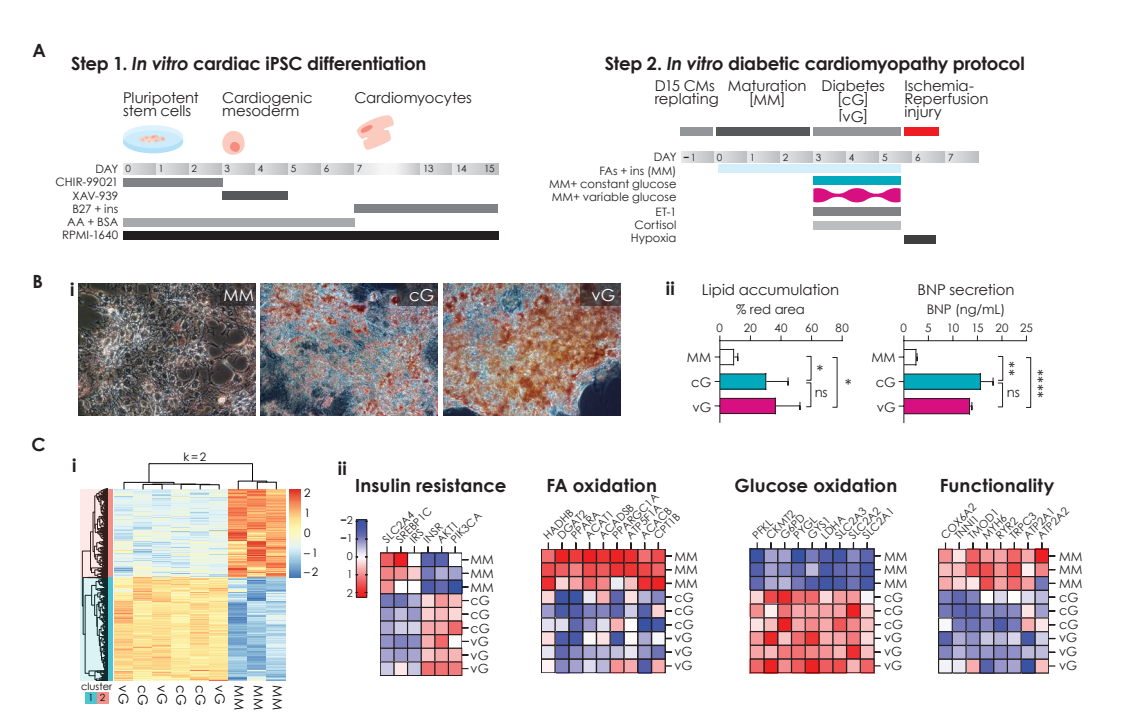

Figure 1. DCM model to evaluate ischemic sensitivity induced by variable vs. constant glycemic stress. A *Step 1, Schematic protocol for small-molecule-directed differentiation from pluripotency into the cardiac lineage (Step 2). Exposing MM-cultured CM to increased glucose and neurohormonal mediators.* ***B*** *Diabetic cardiomyocytes (cG and vG) show increased lipotoxicity (i-ii) characterized by increased intracellular uptake of lipids and enhanced release of the DCM stress marker BNP (ii). Scale Bar=30 µm, n=3.* ***C*** *(i) Differential gene expression classified in clusters among MM, cG and vG conditions, (ii). Key transcriptional hallmarks of the DCM model include insulin resistance, metabolic reprogramming and altered functionality, n=3.*

into cardiac dynamics. Our work with the CardioExcyte 96 system has spanned various areas, including hiPSC-CMs development and physiology, heart disease modeling, and investigating the effects of human COVID plasma on heart cell contractility. Additionally, we have utilized the system to study impedance-based cardiac cell death induced by receptor and ion channel modulators, as well as for physiological validation of stem cell differentiation-modulated heart cells.

Furthermore, in our exploration of the FLEXcyte 96 system, we have discovered several key capabilities and applications. For instance, we aim to utilize it in drug candidate discovery and pharmacological research to assess the effects of compounds on contractility, particularly for cardiac protection. The system has helped us to evaluate the cardiotoxic effects of our pharmaceutical inhibitors within an *in vivo*-mimicking environment. Additionally, it has great potential in facilitating mechanistic studies aimed at understanding the cellular and molecular mechanisms underlying contractility regulation by novel proteins/receptors.

Based on our experiences, I wholeheartedly recommend the CardioExcyte and FLEXcyte systems to any scientist or institution seeking to explore cardiac contractility with precision and efficiency. These systems have undoubtedly enhanced the depth and accuracy of our research, contributing significantly to answering our scientific questions."

In summary, we show that a diabetic cardiomyopathy *in vitro* disease model was successfully validated, and functionally characterized, with the impedance-based CardioExcyte 96 contractility assay.

Specifically, as glycemia variability of DCM is susceptible to contractile impairment, IRI-induced cell death, and metabolic shift, these findings could be fundamental to establish further phenotypic drug screenings for DCM using iPSC-CMs, including myocardial ischemia.

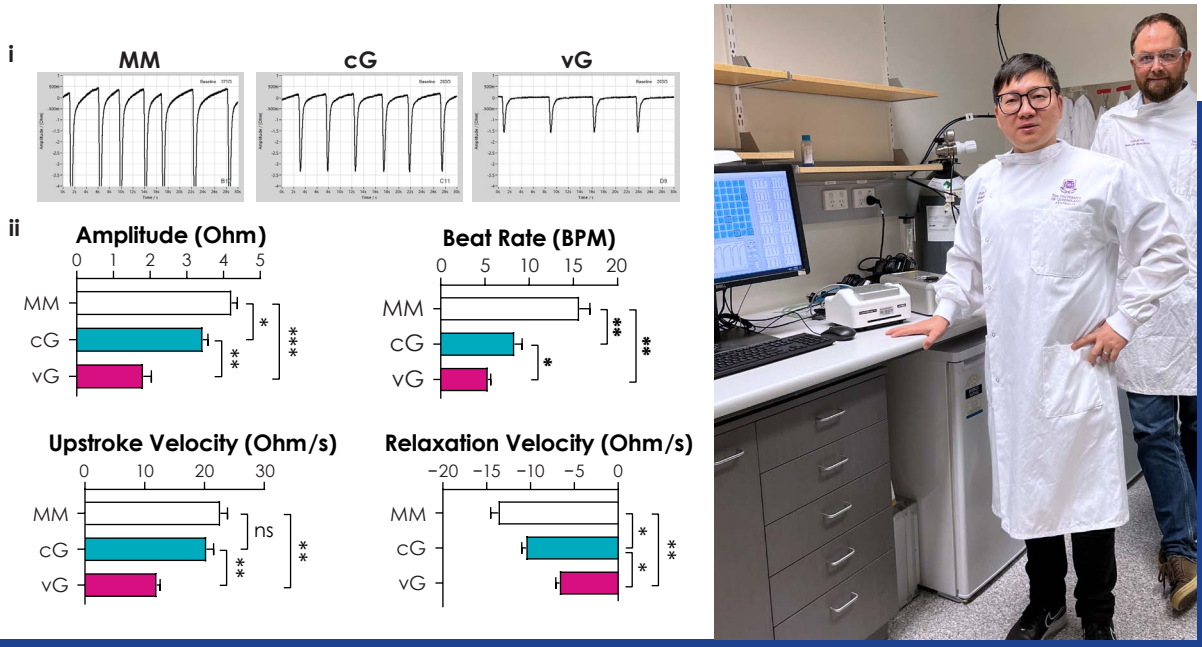

Figure 2. Nick Cao and Nathan Palpant perform measurements on the CardioExcyte 96 at University of Queensland. *The system is routinely used in the lab to investigate diabetes and ischemia models. Diabetic cardiomyocytes have impaired contractile performance shown in raw data traces (i) and bar graphs (ii), displaying decreased contractile amplitude, beat rate, and compromised velocity, each with 3 replicates.*

"I wholeheartedly recommend the CardioExcyte 96 and FLEXcyte 96 to any scientist seeking to explore cardiac contractility with precision and efficiency. These systems have undoubtedly enhanced the depth and accuracy of our research, contributing significantly to answering our scientific questions."

Dr. Yuanzhao (Nick) Cao, The University of Queensland

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Acknowledgments
We thank Nick and Nathan for valuable insights and a wonderful collaboration.

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