Non-invasive impedance monitoring of contractility in Axol Human iPSC-Derived Cardiomyocytes

The ability to monitor cardiomyocyte beat rate in real time is a powerful tool for drug discovery research, particularly when used in conjunction with human induced pluripotent stem cell (iPSC)-derived cells. This offers a physiologically relevant model in which to reliably assess cardiac liability, enabling lead compounds to be identified sooner, thereby reducing the rate of drug attrition and adverse reactions such as proarrythmias. To do this, human iPSC-derived cardiomyocytes (iPSC-CMs) (Axol Bioscience Ltd.) were cultured in a non-invasive impedance monitoring system (xCELLigence®) to assess cardiotoxicity and cell contractility in a 96-well plate format.

Materials and methods

Cardiomyocyte media preparation

Contents of the Axol Cardiomyocyte Maintenance Medium Kit (ax2530-500) were thawed overnight at 4°C. The supplement was then added to the Cardiomyocyte Maintenance Medium to make the complete medium. To make the plating medium, 10% fetal bovine serum (FBS) was added to an aliquot of complete Cardiomyocyte Maintenance Medium.

Plate preparation

50 µL of fibronectin coating solution (10 µg/mL in phosphate buffered saline (PBS) containing Ca^{2+} and Mg^{2+}) was added to each well of the E-Plate® Cardio 96 (ACEA Biosciences, Inc.), and incubated overnight at 4°C. The following day, excess fibronectin was aspirated, 180 µL of pre-warmed plating medium was added to each well and the plate was equilibrated in a 5% CO_2 incubator at 37°C. After 30 mins, the plate was transferred to the xCELLigence® RTCA Cardio in the incubator and background impedance measured as per the manufacturer's protocol.

Cell seeding

Four vials (1x10^6 cells/vial) of Axol Human iPSC-Derived Ventricular Cardiomyocytes (ax2505) were removed from liquid nitrogen storage and placed on dry ice before being thawed rapidly in a 37°C water bath. The vials were swirled constantly and once only a small ice clump was evident, the vials were transferred to a sterile laminar flow hood. The contents of all four vials were transferred to a sterile centrifuge tube containing 6 mL of pre-warmed plating medium. The cell suspension was centrifuged at 200 x *g* for 5 mins and the resulting cell pellet carefully resuspended in 10 mL of fresh plating medium. Cell viability was calculated via trypan blue exclusion using a hemocytometer. Plating medium was used to achieve a final concentration of between 2.4x10^6 and 3.0x10^6 viable cells in a total volume of 18 mL. All media was aspirated from the plate and 180 µL of cell suspension was added to each well resulting in a total of 2.4x10^4 to 3.0x10^4 cells per well. The plate was left in the laminar flow hood for 30 mins to allow the cells to settle and attach after which it was transferred to the xCELLigence® RTCA Cardio in the incubator. Impedance readings were automatically recorded every 30 mins.

Cardiomyocyte maintenance

Once a day, the instrument was paused and the plate transferred to the laminar flow hood in a transfer module at which point 90 µL of medium was removed and replaced with an equal volume of fresh, pre-warmed medium. This was repeated three times before returning the plate to the xCELLigence® RTCA Cardio in the incubator. The iPSC-CMs were cultured using plating medium until cell growth kinetics demonstrated a plateau, indicative of cell coverage of the well and proliferation cessation (approximately 72-96 hrs post-plating). Upon achieving a stable cell index, all plating media was carefully removed from the plate and replaced with complete Cardiomyocyte Maintenance Medium (i.e. serum-free).

Drug application

After approximately 24-72 hrs in serum-free complete medium, a stable synchronous beat rate should be observed. After an additional 72 hrs, a range of concentrations of the β-adrenergic receptor agonist (isoproterenol) or antagonist (carvedilol) were added to selected wells and the iPSC-CMs response evaluated.

Results

Initial impedance readings

Prior to plating, the background reading of all wells within the E-plate® Cardio 96 were found to be within the acceptable range and therefore suitable for the addition of cells and further study evaluation. Post-plating, impedance readings strongly indicated attachment of viable cells to the fibronectin.

Cell growth

During the post-seeding period in plating medium, an increase in impedance (as determined by cell index) was measured in all wells within the plate. This increase was indicative of cell growth and continued viability, with a plateau and cessation of growth reached by 93 hrs (**Figure 1**).

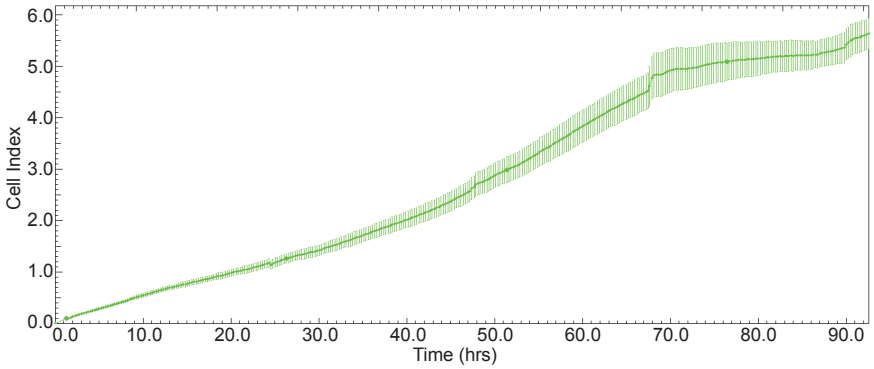

Figure 1: Increased cell index of iPSC-CMs over time. Each well contained 2.4x10^4 cells in plating medium. Exponential cell growth was demonstrated over the initial 93 hrs post-seeding. Cell index (impedance) values plateaued after 93 hrs indicating confluency and cessation of cell proliferation.

Cell contractility and synchronous beating

All wells were monitored for evidence of contractility and synchronicity throughout sub-sequent phases of the study. At 24 hrs post-plating, an indication of primitive cell contractility was observed (**Figure 2a**), with indicative contractility observed by 48 hrs (**Figure 2b**) and synchronous cell contractility at 120 hrs (**Figure 2c**).

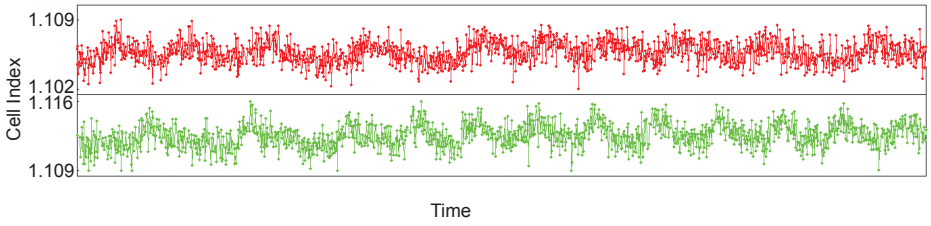

Figure 2a: Primitive asynchronous cell contraction at 24 hrs post-plating in plating medium.

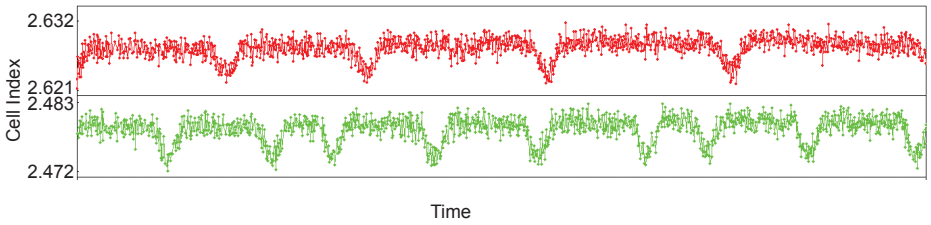

Figure 2b: Indicative evidence of cell contractility at 48 hrs post-plating in plating medium.

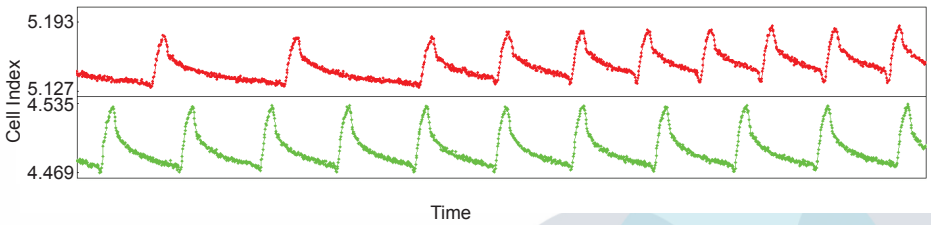

Figure 2c: Indicative evidence of synchronous cell contractility at 120 hrs post-plating (24 hrs in complete Cardiomyocyte Maintenance Medium).

Evaluation of drug effect on cardiomyocyte contractility

A range of isoproterenol and carvedilol concentrations were added to selected wells. The iPSC-CMs responded to the addition of isoproterenol and carvedilol as expected and in a dose-dependent manner (**Figure 3**). This strongly indicates the 'clinical' relevance of these cells and their utility for drug screening applications.

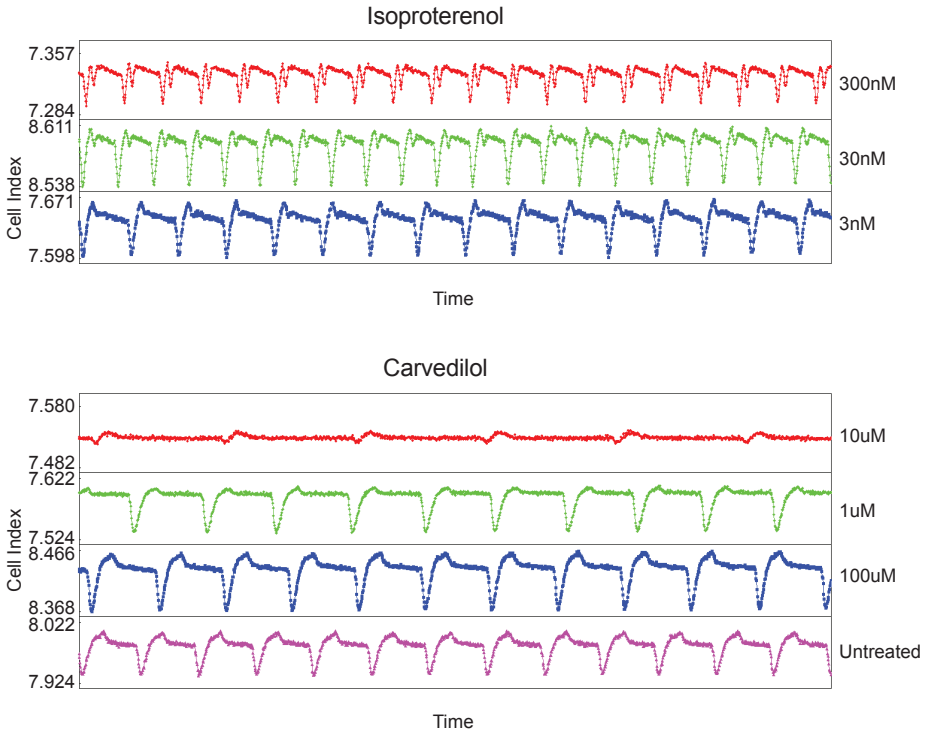

Figure 3: Effect of isoproterenol and carvedilol upon cardiomyocyte contractility.

Conclusions

Axol iPSC-CMs are suitable for use on the xCELLigence® RTCA Cardio instrument when cultured in the E-Plate® Cardio 96 pre-coated with fibronectin. Initial plating of the cells requires the use of a serum-rich plating medium. The iPSC-CMs demonstrate potential for excitation-contraction in the plating medium, but culture of the iPSC-CMs in complete Cardiomyocyte Maintenance Medium (without serum) is conducive for longer-term cell survival and viability. The latter is permissive of excitation-contraction coupled cell contractility, with a clearly defined clinically relevant contractile phenotype. The iPSC-CMs respond to cardiac drugs and therefore have potential for use in cardiotoxicity and cardiomyocyte-pharmacology studies.

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