

IntraCell[♥]

Application protocol



Action Potential (AP) MEA Recordings from Human iPSC-derived Cardiomyocytes After Acute and Chronic Exposure to Drugs

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1. Introduction

This application protocol describes the full procedure to obtain recordings of Action Potentials (APs) from human iPSC-derived cardiomyocytes (hiPSC-CMs) cultured on microelectrode arrays (MEA) using the unique capabilities of IntraCell.

While this protocol is compatible with a large variety of cardiac cellular models, the following details and the resulting data were obtained with cultured iCell® Cardiomyocytes from FUJIFILM Cellular Dynamics, Inc. (FCDI).

These cells are highly purified human cardiomyocytes derived from induced pluripotent stem (iPS) cells using FCDI's proprietary differentiation and purification protocol. iCell Cardiomyocytes are a mixture of spontaneously electrically active atrial-, nodal- and ventricular-like myocytes with typical biochemical, electrophysiological and mechanical characteristics and expected responses upon exposure to exogenous agents. These features make iCell Cardiomyocytes a reliable platform for testing cardiac biology in basic research as well as several areas of drug development.

Electrophysiological recordings are obtained using MEAs and acquisition systems from Multi Channel Systems MCS GmbH (<https://www.multichannelsystems.com/>) (MCS), which allow for recording activity from several types of electrogenic cells plated on optically transparent substrates. **The recording of APs with high signal-to-noise ratio is achieved thanks to the laser technology of Foresee Biosystems implemented in IntraCell. IntraCell enables recordings of high-quality APs by applying a proprietary laser optoporation protocol to the cells cultured on the MEA. The extraordinary feature of laser optoporation is its negligible invasiveness. Cells are not damaged or affected by the process and can be maintained in culture for as long as allowed by the used cellular model. Moreover, laser optoporation and AP recordings can be repeated on the same cells several times over many weeks.**

It's worth noting that IntraCell works in combination with all recent MEA systems offered by MCS, thus including:

- MEA2100-Mini-60-System
- MEA2100-Mini-120-System
- MEA2100-Lite-System
- MEA2100-256-System

This application protocol describes how to manipulate iCell Cardiomyocytes from FCDI and to obtain APs after acute and chronic exposure to drugs. The exemplary results were obtained using a MEA2100-Mini-System from MCS, but similar results may be obtained with other MEA systems from MCS.

2. Required Materials

ITEM	PROVIDER	CATALOGUE #
iCell Cardiomyocytes kit, 11713	FCDI	R1105
MEA plates	Multi Channel Systems MCS	
Fibronectin	Roche Applied Science	11051407001
Sterile water	Multiple Vendors	
Phosphate Buffered Saline w/o Ca^{2+} and Mg^{2+} (D-PBS)	Sigma-Aldrich	D8537
IntraCell	Foresee Biosystems SRL	
MEA2100-Mini-System	Multi Channel Systems MCS	
FB_Alps	Foresee Biosystems SRL	
Multi Channel Experimenter	Multi Channel Systems MCS	

Table1: List of materials.

3. Cell culture

Cardiac cells must be plated on the selected MEA according to the protocols provided by the cell supplier.

For optimal performance, please refer to the manufacturers recommendations, as well as application note supported by FCDI in partnership with Multi Channel Systems MCS and NMI available here:

- 1) https://www.fujifilmcdi.com/wp/wp-content/uploads/2021/10/FCDI_iCellCardiomyocytes_UG_Final.pdf
- 2) <https://www.nmi-tt.de/files/daten/PDF/PDF%20PS/PDF%20Electrophysiology/Application%20Protocol%20Electrophysiology/NMI-%20iCell%20Cardiomyocytes%20Application%20Protocol%20Multiwell-MEA.pdf>

The whole procedure of culture preparation is briefly described below. It is an optimized protocol for culturing iCell Cardiomyocytes (see the Table1) on MEA plates; however, we strongly recommend following the instructions from the cell's provider and adapt cell's culture conditions according with your needs.

1. Place the MEA plate within a 60 mm cell culture dish. Fill a 35 mm cell culture dish with sterile water and place it within the 60 mm cell culture dish, beside the MEA. This will provide a humid environment to prevent droplet evaporation during the coating procedure and cell attachment on the MEA described below.

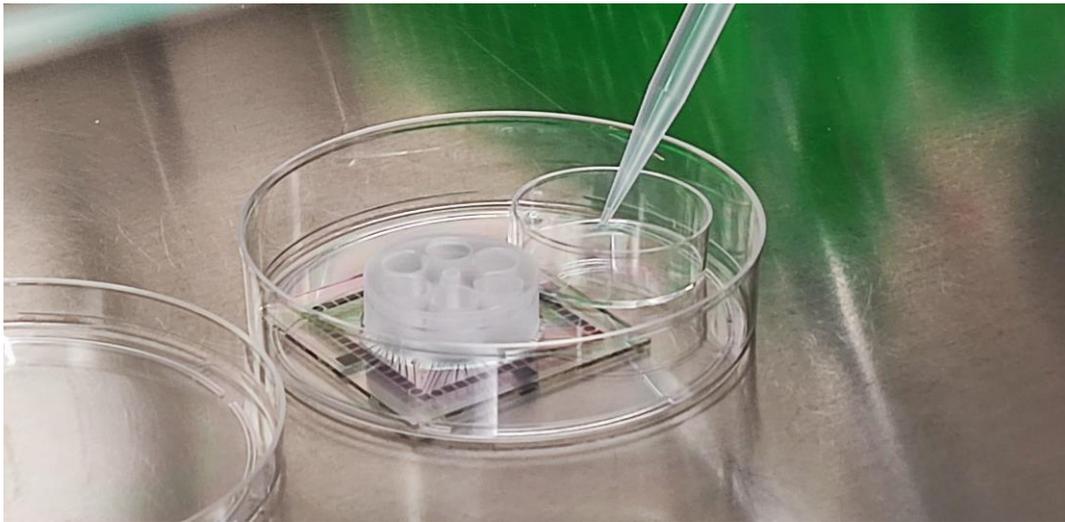


Figure 1: System configuration during coating and cell seeding procedure to avoid droplet evaporation.

2. Dispense a 8 μl /well droplet of 50 $\mu\text{g/ml}$ fibronectin solution over the recording electrode area of each well of the MEA plate and incubate the MEA plate in a cell culture incubator at 37°C, for 1 hour.
3. Thaw iCell Cardiomyocytes into Plating Medium, according to the provider's protocol.
4. Dispense a 8 μl droplet of iCell Cardiomyocytes cell suspension (approximately 16,000 cells) on the centre of the MEA electrodes layout and incubate MEA plate in a cell culture incubator at 37°C, for 1 hour. This procedure is flexible and suitable for all types of MEA devices, ranging from standard single-well up to multiwell plates with 6 or 9 wells. Exemplary MEA plates are reported in Figure 2.

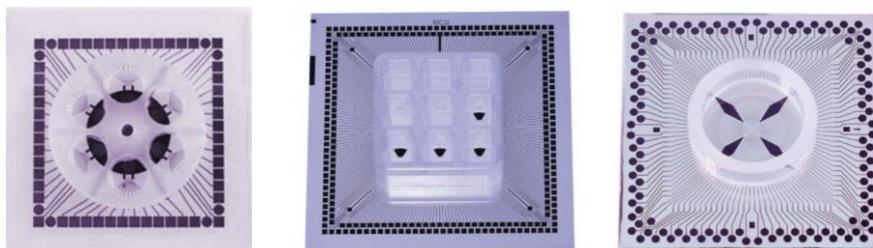


Figure 2: MEA devices from Multi Channel Systems GmbH.

5. Carefully add Plating Medium to the MEA plate (1.5 ml, 350 μl /well, 250 μl /well for standard single-well, 6-well and 9-well plate, respectively) and replace the MEA in the incubator. On Day 2 post-plating, replace 100% of the Plating Medium with Maintenance Medium equilibrated in a 37°C water bath. Ensure not to touch or disturb the attached cardiomyocytes.
6. Maintain the cardiomyocytes in the MEA devices replacing 50% of the spent medium with Maintenance Medium every Monday, Wednesday, and Friday.
7. Perform MEA recordings between 10 and 14 days after the initial plating.

4. Baseline recording

When cells acquire regular spontaneous activity (10 - 14 days post-plating), the electrophysiological activity can be recorded with the following steps:

1. Take the sample from the incubator and replace 50% of spent medium with the Maintenance Medium following the supplier's protocol; then put it back inside the incubator.
2. Wait at least 2-4 hours from the medium replacement, allowing the sample to stabilize.
3. Clean the MEA head stage with pure ethanol.
4. Take the sample from the incubator and mount it on the MEA head stage under sterile conditions.
5. Place a compatible cap on the MEA ring for preserving sterility, as shown in Figure 3.

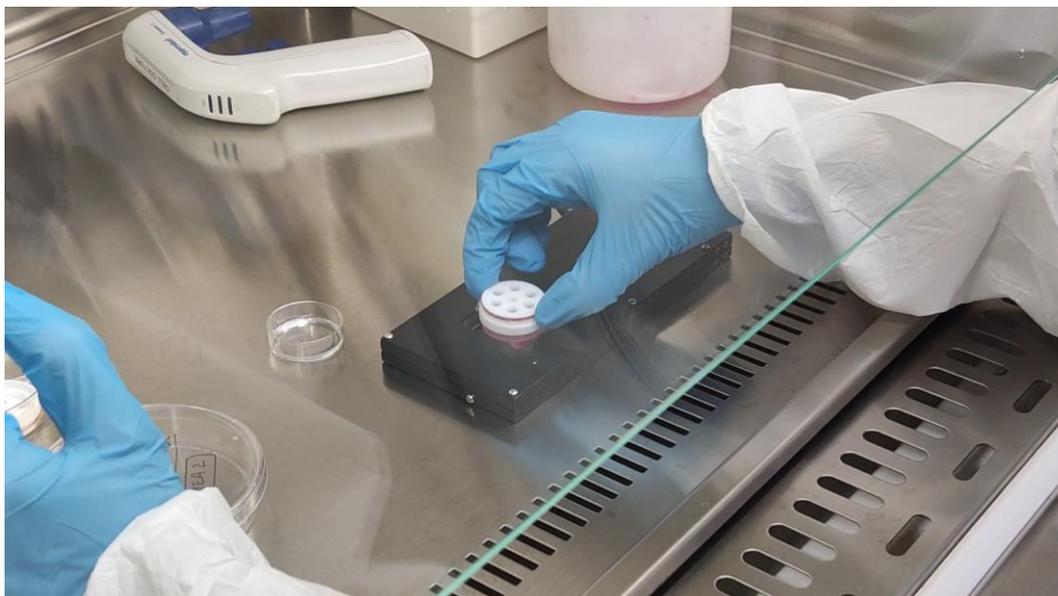


Figure 3: the operator places the cap on the top of the MEA. The whole procedure is performed under sterile conditions.

6. Move the MEA headstage from cell hood and place it on top of IntraCell, as shown in Figure 4. Open the lid of IntraCell and place the MEA headstage in the dedicated socket.

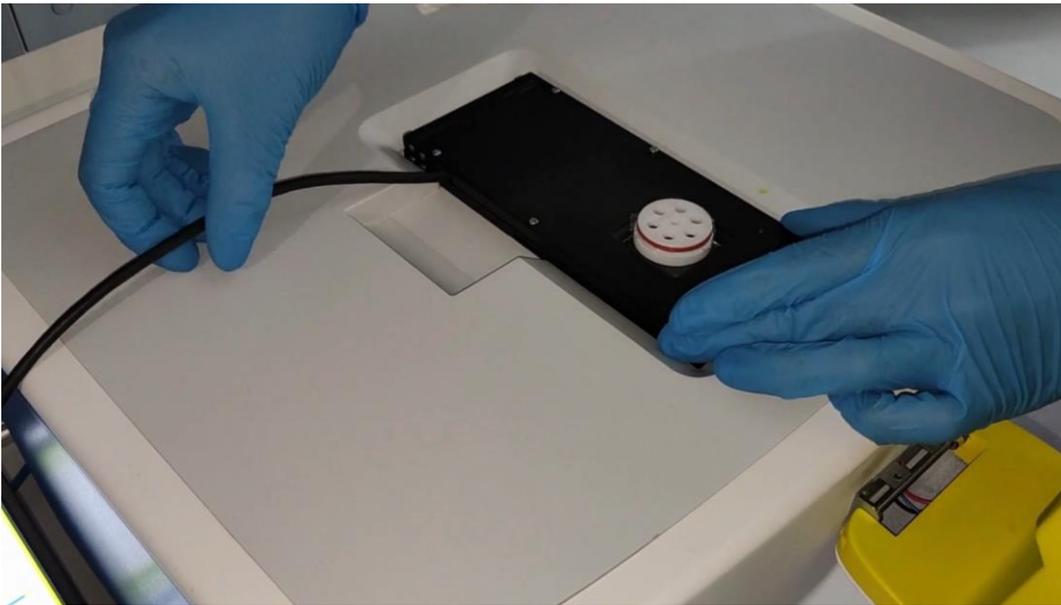


Figure 4: The operator completes the configuration of the combined systems: IntraCell plus the MCS MEA headstage.

7. Close the lid of IntraCell.
8. Open the software FB_Alps: the main window will appear on the screen (Figure 5) showing:
 - a. Information Card
 - b. Camera View
 - c. MEA layout and control buttons
 - d. Laser control panel
 - e. Scan settings panel
 - f. Imaging settings panel
 - g. Action panel
 - h. Cheat sheet

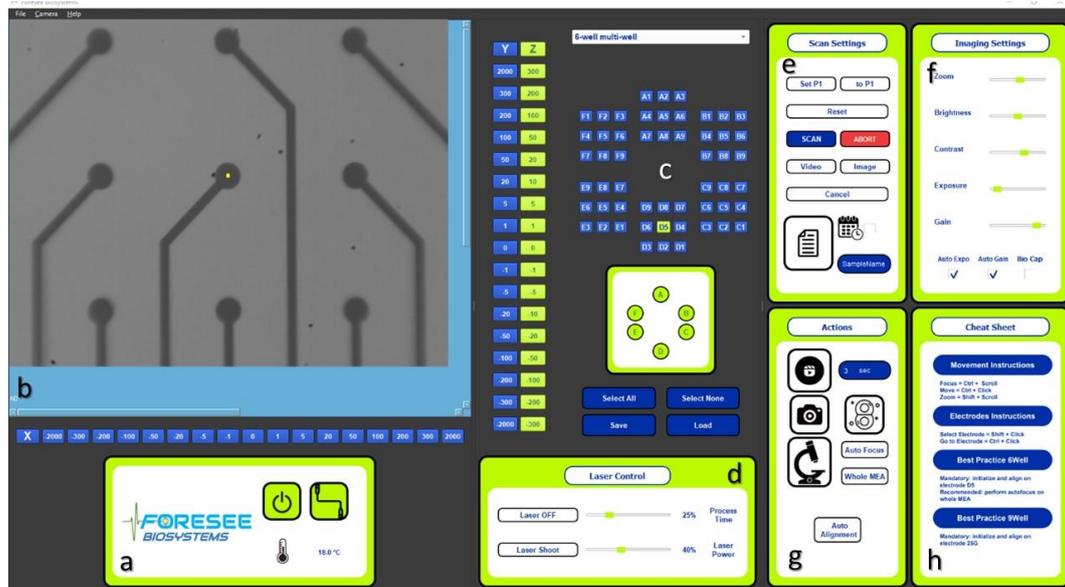


Figure 5: Software FB_Alps: a. Information card; b. Camera view; c. MEA layout and control buttons; d. Laser control; e. Scan settings; f. Image settings; g. Action panel; h. Cheat Sheet.

9. Select the MEA scheme via the MEA layout dropdown button.
10. To adjust the focus: use the Z-bar in the camera view, or ctrl + wheel, and move along the Z-axis to reach the desired focus. Autofocus button can also be used to obtain an automatically focused MEA image. If the user is not satisfied with the result, the focus can also be manually modified after the procedure is completed.
11. Move along the MEA surface in the camera view by pressing Ctrl + Left Mouse or using the X- and Y-bars and set the bullseye (yellow dot) on the reference electrode. Please refer to the IntraCell user manual to know the reference electrodes of each compatible layout.
12. Before starting any procedure, press Set P1 button while on the reference electrode.
For each well to analyse, press the Autofocus button to adjust the focus, and the Auto-alignment button to set the central electrode. Alternately, press the Whole MEA button to perform the Autofocus – Auto-alignment procedure automatically on the entire MEA.
13. Select the electrodes on the MEA scheme by clicking Shift + Left click. If you want to scan the entire MEA press on Select all.
14. Turn on the Laser using the relative button in the Laser panel.
15. Click on the Scan button to perform laser optoporation on the selected electrodes, note that a successful optoporation is obtained only if the MEA surface is in focus in the camera view. Please remember to start MEA DAQ acquisition from the Multi Channel System software before starting the optoporation process.
16. Use the MCS interface to visualize AP recordings on each electrode selected for optoporation, as shown in Figure 6. Please refer to the manufacturer manual for more details.

17. (OPTIONAL) IntraCell may be used to optically inspect the cell culture and to acquire images or videos of contracting cells and perform a contractility analysis. For further information, please refer to the IntraCell user manual.
18. Other procedures can be found in the IntraCell user manual.

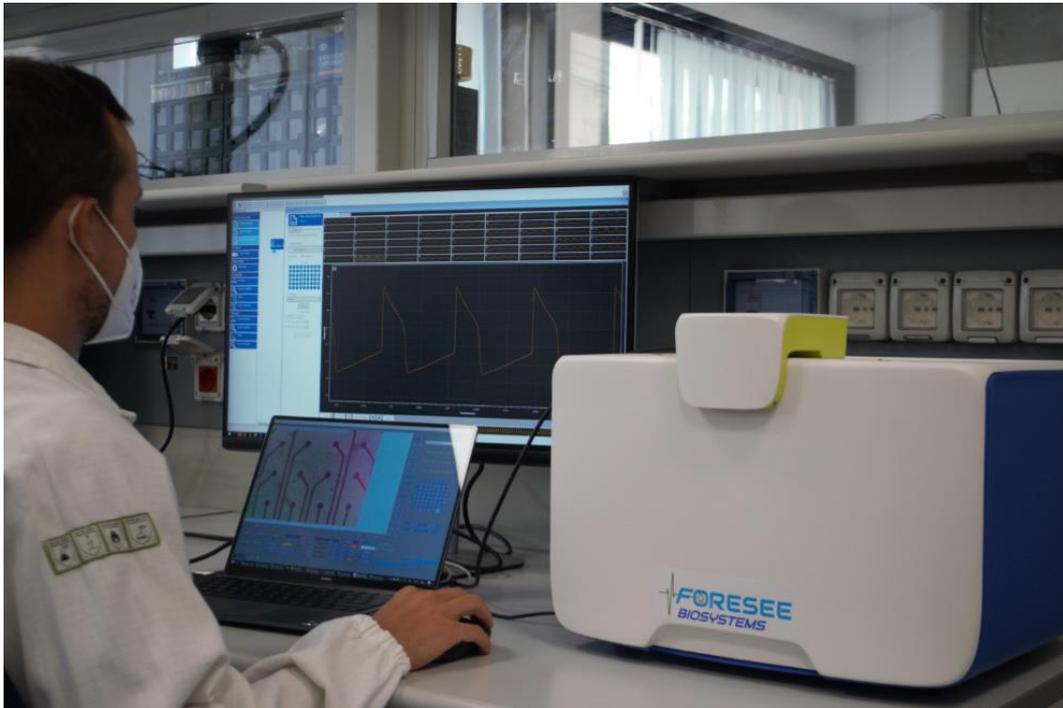


Figure 6: the user performs AP recordings exploiting IntraCell and FB_Alps.

Thus, the user can use IntraCell capabilities to perform single or parallel laser cell optoporation over the electrodes to obtain APs from several cell. Immediately after the laser pulse, transient nanopores are opened on the cell membrane, allowing to switch from field potential to APs with no side effects on the spontaneous activity of the cell, as shown in the exemplary recording in Figure 7.

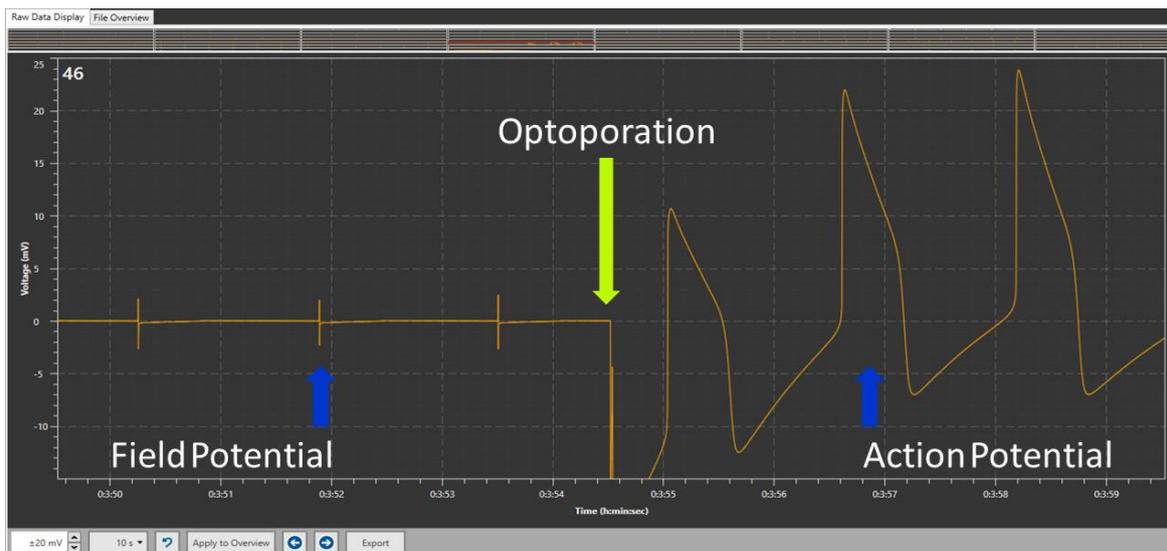


Figure 7: transition from the extracellular field potential to the intracellular action potential recording immediately after the laser optoporation.

Using the automated laser scan technology over the whole 6-well MEA, you will be able to record intracellular APs from each electrode, as shown in Figure 8.

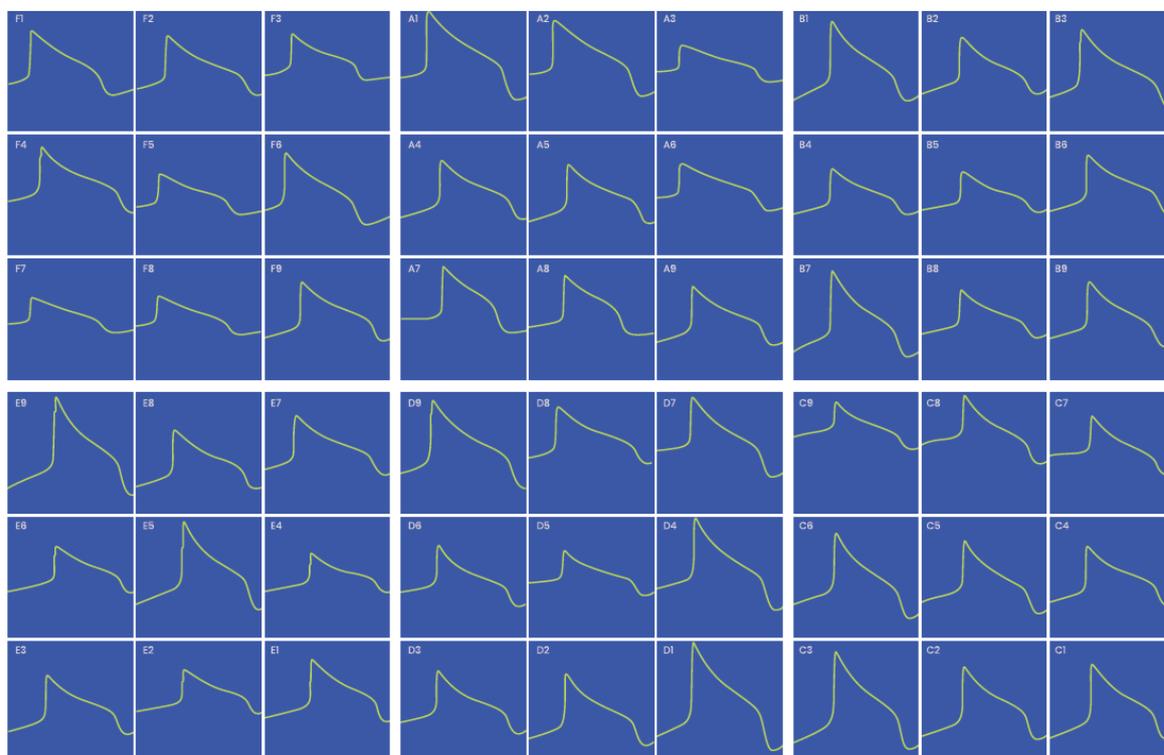


Figure 8: AP recordings over the whole 6-well MEA after a complete process of laser scan optoporation.

5. Acute drug treatment

After the recording of baseline APs, the user can apply molecules of interest such as Verapamil, Nifedipine, Terfenadine, and E4031. The user needs to prepare the solution with the test molecule(s), then open the lid of IntraCell and gently inject the solution in the cell culture medium with a micropipette (Figure 9). After molecule administration, it is good practice to gently mix the cell medium with the micropipette and then wait 5-10 minutes before resuming measurements, according to the expected effects of the molecule under investigation.

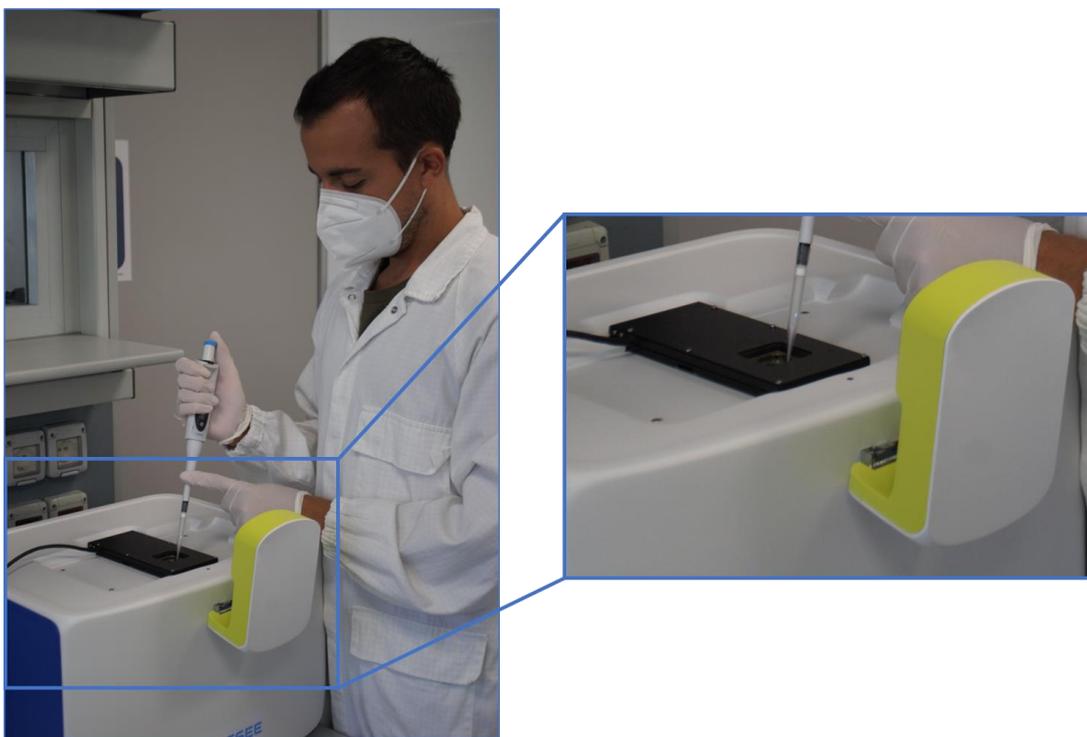


Figure 9: drug injection within the cardiac cell culture.

Immediately after administration, it is possible to analyse potential adverse **acute** effects of the molecule on the cardiac AP. IntraCell allows for the precise detection of changes of the AP shape related to drug adverse-effects over many cardiac cell models, as shown in Figure 10. AP duration prolongation or Early afterdepolarizations (EADs) occurrence can be monitored with IntraCell. Once the recording of the acute response is completed, the sample can be cleaned and placed back into the incubator.

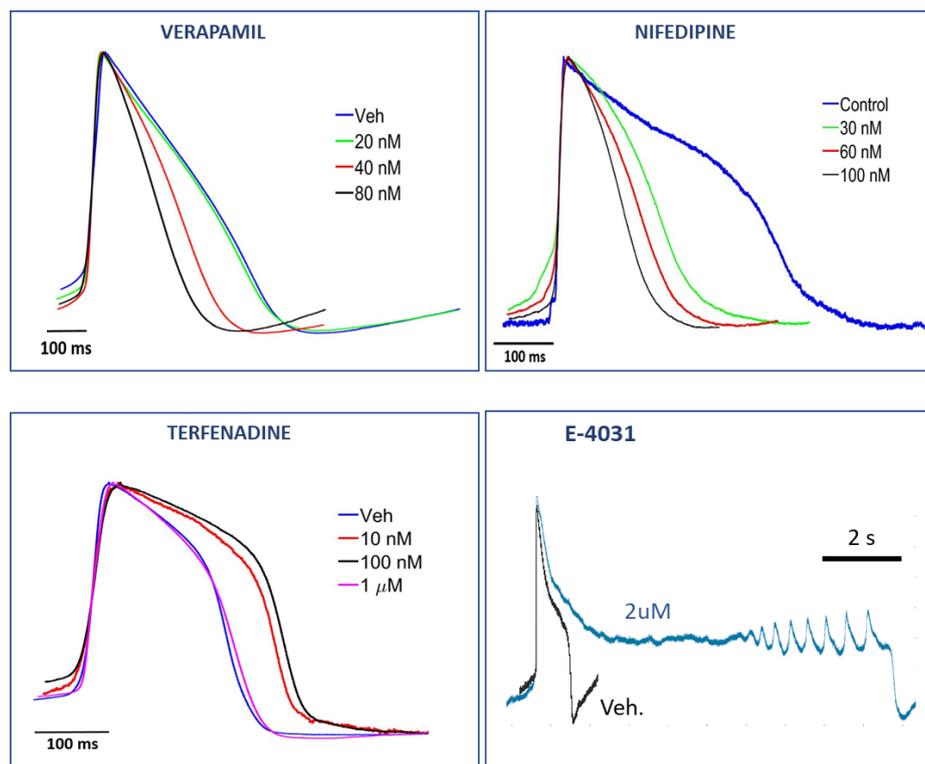


Figure 10: AP recordings after acute exposure to different drugs.

After the detection of the acute adverse effects, the user has the possibility to go on and assess the chronic adverse effects of a drug after long-term exposure. Indeed, thanks to the lack of any harsh effect on the cell viability, the system can measure APs from the same sample multiple times during a prolonged time range (for up to 45 days).

Therefore, it is possible to remove the MEA headstage from IntraCell and place it under a sterile cell hood. Once the MEA device has been removed from the headstage, the user must clean the glass surface of the MEA device (external to the culture ring), with a 75% ethanol/water solution, using a paper stripe, to prevent contaminations or remove cell medium that might have been spilled on the MEA glass surface. Now, the MEA can be placed back in the incubator and a second cycle of measurements can be later performed following the instructions below.

6. Chronic compound dosing

IntraCell is the first tool for the analysis of adverse effects after long term drug exposure and thus for monitoring **chronic effects** on AP of *in vitro* hiPSC-CMs. Therefore, we developed a robust protocol of long-term maintenance and AP recordings over several weeks from the hiPSC-CMs, as shown in Figure 11.

It is worth noting that IntraCell enables AP recordings for multiple times from the same cellular sample. IntraCell, together with the long-term protocol, offers an unprecedented solution for preserving both

biological samples and consumables over several measurement sessions, thus reducing time and cost of your research activities.

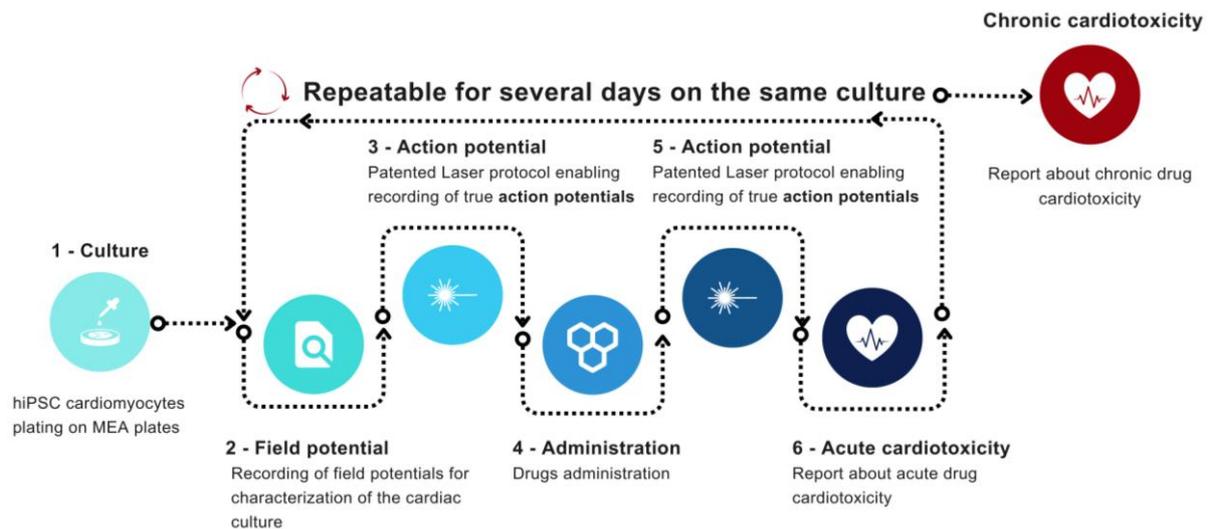


Figure 11: chronic AP recordings cyclic procedure.

It is highly recommended to follow the instructions of this application protocol (see section 3 and 4) to perform optimal electrophysiology experiments. However, the success of the procedure will depend also on the user's skills to handle sensitive cell cultures such as the cardiac one, critical for example to preserve cell's adhesion on the MEA devices. Figure 12 shows a panel of results about the detection of Pentamidine chronic effects with 2 concentrations (0.5 μ M and 1.5 μ M) and the recovery of cardiomyocytes after drug washout. The experiment was carried out using 2 6-well MEAs, one for each concentration, monitoring the same samples over a long time-window.

After the application of the laser scan over all the electrodes of the MEAs, it's possible to obtain APs from each of these electrodes. Then, data are averaged to obtain a mean AP for each day of treatment. One can apply the same procedure with more samples, to parallelize many experiments.

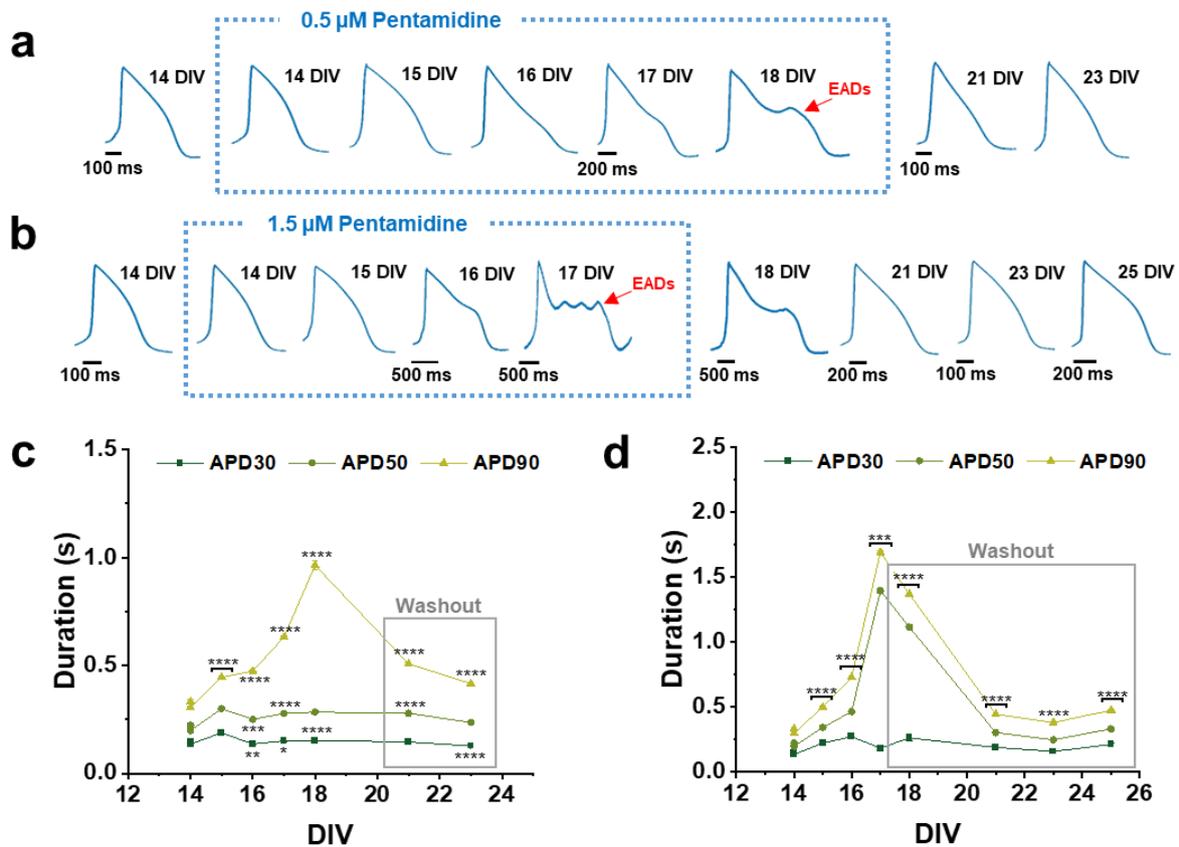


Figure 12: detection of Pentamidine chronic effects at 2 concentrations, (a) 0.5 μM and (b) 1.5 μM , over a long-time window, with early afterdepolarization events (EADs). (c) and (d) show respectively APD at 30%, 50% and 90% of repolarization at 0.5 μM and 1.5 μM .

7. Contacts

Foresee Biosystems SRL

info@foreseebiosystems.com

Dr Michele Dipalo

Michele.dipalo@foreseebiosystems.com

Dr Giovanni Melle

Giovanni.melle@foreseebiosystems.com

Giuliana Orlandini

Giuliana.orlandini@foreseebiosystems.com

Dr Giuseppina Iachetta

Giuseppina.iachetta@foreseebiosystems.com