Application Note

Channels: hERG, $Na_V1.5$, $Ca_V1.2$,
Cells: human iPS-derived cardiomyocytes
Tools: Patchliner, SyncroPatch 96, SyncroPatch 384PE, CardioExcyte 96

Combining automated patch clamp, impedance and EFP of hiPSC-CMs

The electrophysiology team at Nanion Technologies GmbH, Munich.
Cellartis® Cardiomyocytes kindly provided by Takara Bio.

Summary

Cardiomyocytes derived from human induced pluripotent stem cells (hiPSC-CMs) are gaining interest in cardiac safety screening. Given their recapitulation of native behavior, availability, ease of use and standardized production, they are likely to provide a viable alternative to acutely isolated cardiomyocytes to assess the pro-arrhythmic potentials of drug candidates. In 2013 the Comprehensive In-vitro Proarrhythmia Assay (CiPA) was introduced to provide a more complete assessment of pro-arrythmic risk by evaluating and implementing currently available high throughput methods[1,2] and evaluating the potential use of hiPSC-CMs as a model system for cardiac safety testing. Until now, drug safety testing has focussed on interaction with the hERG channel and QT prolongation which can lead to potentially fatal torsades de pointes (TdP). Although this approach has been largely successful in preventing new drugs reaching the market with unexpected potential to cause TdP, it is also possible that potentially valuable therapeutics have failed due to this early screening. The CiPA initiative has proposed an expansion of patch clamp assessment beyond hERG to include, e.g. $Na_V1.5$ and $Ca_V1.2$. In addition, techniques such as multi-electrode array (MEA) and impedance are being thoroughly evaluated as complementary techniques to patch clamp.

Here we present data recorded using the automated patch clamp platforms, the Patchliner, SyncroPatch 96 and SyncroPatch 384PE on Cellartis® Cardiomyocytes (Takara Bio Europe Cat nr. Y10075). Recordings of $Na_V1.5$ and $Ca_V1.2$ are shown. Impedance and EFP recordings were also performed using the CardioExcyte 96, and the effects of verapamil and sotalol are shown.

Download more Application Notes from www.nanion.de

Results

Voltage-gated Na^+ (Na_V) and Ca^{2+} (Ca_V) currents were recorded on the Patchliner using Cellartis® Cardiomyocytes (Figure 1).

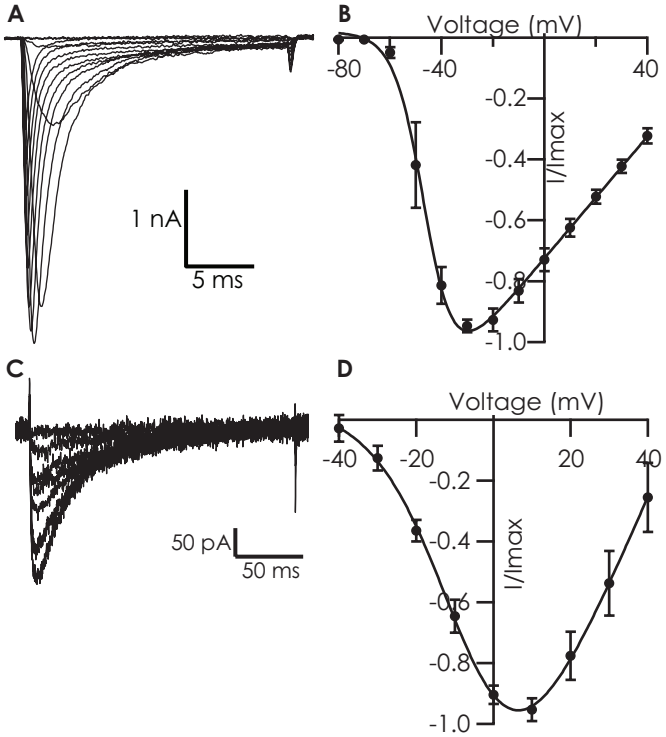

Figure 1: Typical recordings from Cellartis Cardiomyocytes recorded on the Patchliner. A Na_V currents in response to increasing voltage steps. **B** Corresponding current-voltage plot for an average of 7 cells. A Boltzmann fit revealed a Vhalf of activation of -46 mV. **C** Ca_V currents in response to increasing voltage steps. **D** Corresponding current-voltage plot for an average of 18 cells. A Boltzmann fit revealed a Vhalf of activation of -5.8 mV.

Application Note

Cellartis Cardiomyocytes could be captured on the Patchliner NPC-16 chips with a good success rate (see Table 1). Both Na_V and Ca_V currents could be recorded in approximately 70% of cells captured (Table 1; Figure 1). Parameters such as seal resistance (RSeal) and current size for Na^+ and Ca^{2+} are given in Table 2. A current-voltage plot for Na_V for an average of 7 cells is shown in Figure 1B. A Boltzmann equation was fitted to the normalized data revealing a Vhalf of activation of -46 mV, in good agreement with the literature value for the cardiac sodium channel, $Na_V1.5$[3,4]. A current-voltage plot for Ca_V for an average of 18 cells is shown in Figure 1D. A Boltzmann equation was fitted to the normalized data revealing a Vhalf of activation of -5.8 mV, in good agreement with the literature value for the cardiac calcium channel, $Ca_V1.2$[5,6].

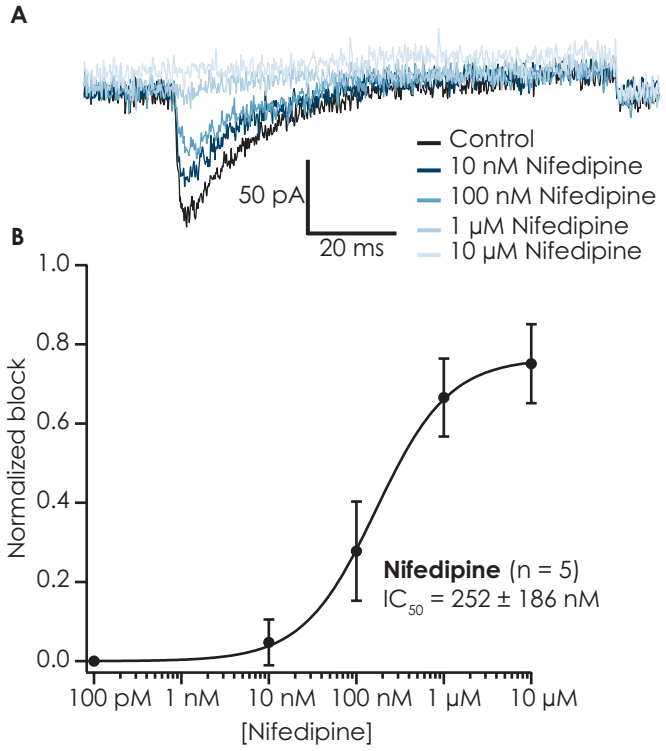

Figure 2: Effect of nifedipine on $Ca_V1.2$ recorded from Cellartis Cardiomyocytes on the Patchliner. A Raw current traces of $Ca_V1.2$ in control conditions (black) and inhibition by increasing concentrations of nifedipine (blue). **B** The concentration response curve (normalized to maximum block) for nifedipine for an average of 5 cells. The average concentration response curve was fitted with a standard Hill-equation which revealed an IC_{50} = 252 ± 186 nM (n = 5).

Capture rate (%)	% cells with Na_V (> -50 pA)	% cells with Ca_V (> -50 pA)
58 (28/48)	**71** (20/28)	68 (19/28)

Table 1: Success rates for cell capture and cells expressing Na_V and Ca_V currents from 1 experimental day. 6 experiments were performed using a total of 3 chips, therefore 48 potential sites on the chip were available and 28 cells were captured (with seal resistance > 150 MΩ) resulting in a success rate of 58% for capture. Of the cells captured, 71% showed Na_V current >-50 pA and 68% showed Ca_V current > -50 pA. Average RSeal, Cm, Rs, Na_V and Ca_V current values are given in Table 2.

The Ca_V current recorded on the Patchliner was blocked by increasing concentrations of nifedipine (Figure 2). The concentration response curve for an average of 5 cells is shown in Figure 2B. The concentration response curve was fit with a Hill equation revealing an IC_{50} = 252 ± 186 nM (n = 5) in good agreement with calcium current block in guinea pig ventricular myocytes[7].

RSeal (MΩ)	Cm (pF)	Rs (MΩ)	I_{Na} at -30 mV (nA)	ICa at 10 mV (pA)
976 ± 144 (28)	37 ± 6 (28)	6.0 ± 0.9 (28)	-5.4 ± 1.5 (7)	-157 ± 24 (18)

Table 2: Average cell parameters for Cellartis Cardiomyocytes. Shown are values for seal resistance (RSeal), cell capacitance (Cm) and series resistance (Rs) for Cellartis Cardiomyocytes captured with seal resistance > 150 MΩ. Na_V current at -30 mV and Ca_V current at 10 mV is also shown. Number of cells shown in brackets. Note that the average current is taken from the IV curves and not all cells which had a detectable Na_V current were used for the IV analysis.

Cellartis Cardiomyocytes were also used on the high throughput automated patch clamp devices, the SyncroPatch 96 and SyncroPatch 384PE. Figure 3 shows the Na_V and Ca_V currents from Cellartis Cardiomyocytes recorded in a pharmaceutical company screening laboratory.

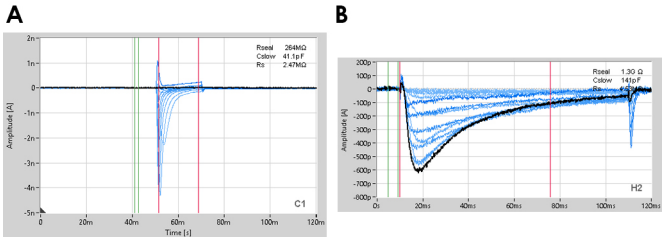

Figure 3: Na_V and Ca_V currents from Cellartis Cardiomyocytes recorded on the SyncroPatch 96 and SyncroPatch 384PE. A Raw current traces of $Na_V1.5$ in response to increasing voltage steps recorded on the SyncroPatch 96. **B** Raw current traces of $Ca_V1.2$ in response to increasing voltage steps recorded on the SyncroPatch 384PE.

Nanion Technologies GmbH
Gabrielenstr. 9
80636 Munich, Germany

phone +49 89 218997972
fax +49 89 218997960
www.nanion.de • info@nanion.de

Application Note

Figures 4 and 5 show screenshots of the SyncroPatch 384PE software during a recording using three different cell types in one experiment, CHO cells expressing $Na_V1.5$ or $Ca_V1.2$ or Cellartis Cardiomyocytes. $Na_V1.5$ expressed in CHO cells and in Cellartis Cardiomyocytes was blocked by tetracaine (Figure 4), $Ca_V1.2$ expressed in CHO cells and in Cellartis Cardiomyocytes was blocked by nifedipine (Figure 5). This confirms that Cellartis Cardiomyocytes are suitable for use on high throughput automated patch clamp devices and cardiac ion channel currents, $Na_V1.5$ and $Ca_V1.2$, can be reliably measured.

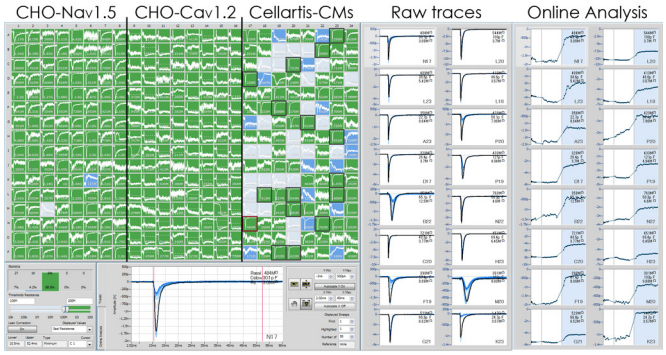

Figure 4: Cell lines and Cellartis Cardiomyocytes recorded on the SyncroPatch 384PE in one experiment. Shown are raw current traces recorded from CHO cells expressing $Na_V1.5$ (left), $Ca_V1.2$ (middle) and Cellartis Cardiomyocytes in response to a single voltage step. $Na_V1.5$ was blocked by tetracaine at different concentrations. Sixteen examples of raw traces and online analysis of the Cellartis Cardiomyocytes is shown on the right.

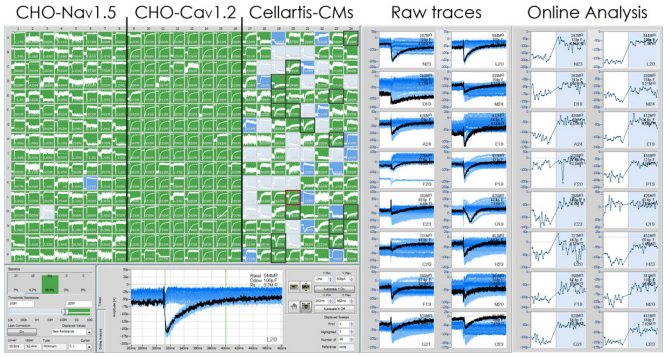

Figure 5: Cell lines and Cellartis Cardiomyocytes recorded on the SyncroPatch 384PE in one experiment. Shown are raw current traces recorded from CHO cells expressing $Na_V1.5$ (left), $Ca_V1.2$ (middle) and Cellartis Cardiomyocytes in response to a single voltage step. $Ca_V1.2$ was blocked by nifedipine at different concentrations. Sixteen examples of raw traces and online analysis of the Cellartis Cardiomyocytes is shown on the right.

The CardioExcyte 96 is a hybrid device for recording impedance (contractility) and extracellular field potential (EFP) from a beating monolayer of cells. The technique is non-invasive and the data acquired is complementary to patch clamp electrophysiology. Cellartis Cardiomyocytes were grown on the NSP-96 plates for approximately 14 days to ensure a synchronously beating monolayer of cells before experiments were begun. Figure 6 shows the impedance signal (left) and the EFP signal (right) in control conditions and in increasing concentrations of the calcium channel blocker, verapamil. Interestingly, verapamil has been shown to also block hERG at low concentrations[8]. Verapamil has differential effects on the cardiac action potential, prolonging the cardiac action potential at low concentrations and shortening it at higher concentrations[9]. These differential effects presumably arise due to the action of verapamil on multiple cardiac ion channels.

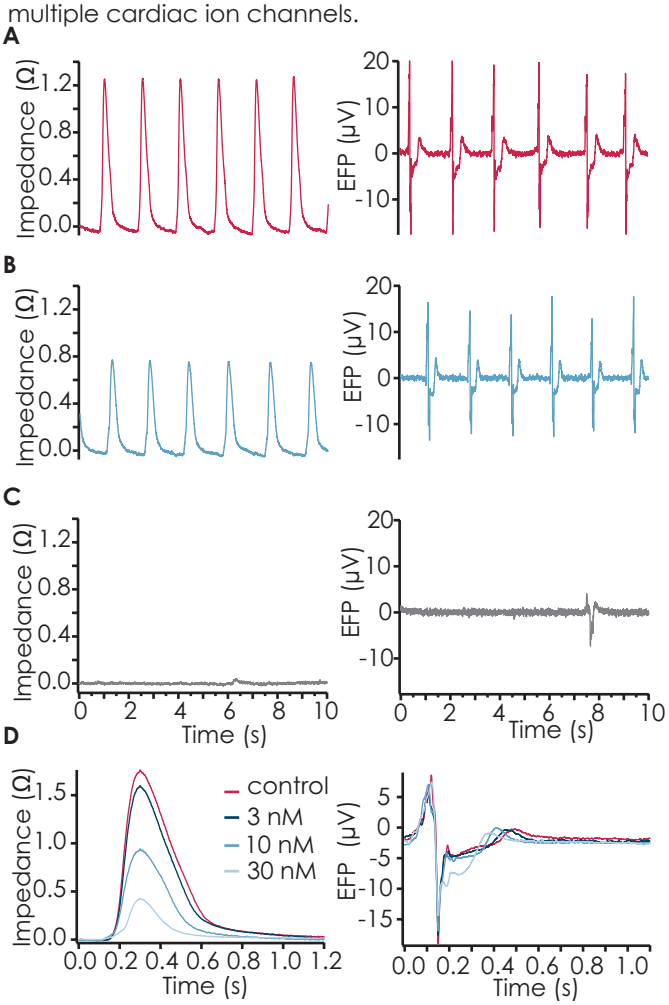

Figure 6: Effects of verapamil on impedance (left) and EFP (right) signals of Cellartis Cardiomyocytes on the CardioExcyte 96. Impedance (left) and EFP signals (right) in control conditions **(A)**, 10 nM verapamil **(B)** and 100 nM verapamil **(C)** are shown. The mean beats for impedance (left) and EFP (right) are shown in panel **(D)**.

Nanion Technologies GmbH
Gabrielenstr. 9
80636 Munich, Germany

phone +49 89 218997972
fax +49 89 218997960
www.nanion.de • info@nanion.de

Application Note

Using the CardioExcyte 96, we found that at low concentrations, verapamil caused an increase in beat rate in both impedance and EFP modes and at higher concentrations, completely disrupted beating of the monolayer. This is consistent with effects on multiple ion channels.

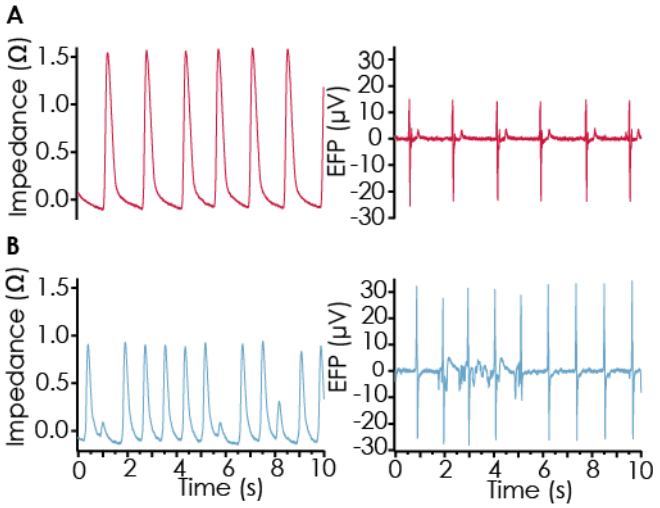

Figure 7: Effects of sotalol on impedance (left) and EFP (right) signals of Cellartis Cardiomyocytes on the CardioExcyte 96. Impedance (left) and EFP signals (right) in control conditions **(A)** and 1 μM sotalol **(B)** are shown. Irregular beating can be observed in the presence of sotalol.

Sotalol is a benzenesulfonamide Class III antiarrhythmic agent known to block the hERG channel[10]. Sotalol has been shown to cause beating irregularities using impedance measurements of mouse stem cell-derived cardiomyocytes[11]. Using the Cellartis Cardiomyocytes, we found that sotalol also caused irregular beating in both impedance and EFP modes, indicated by the secondary beats that could be detected (Figure 7).

In conclusion, Nanion Technologies offers automated patch clamp systems and an impedance/EFP platform which are compatible with recording from the hiPSC-cardiomyocytes, Cellartis Cardiomyocytes from Takara Bio. Voltage-gated Na^+ and Ca^{2+} currents could be recorded from these cells on the Patchliner, SyncroPatch 96 and SyncroPatch 384PE. The Na_V current was blocked by tetracaine and the Ca_V current was blocked by nifedipine. On the CardioExcyte 96, verapamil, a L-type Ca^{2+} channel and hERG blocker, completely disrupted beating at higher concentrations. The hERG blocker, Sotalol caused irregular beating in impedance and EFP modes as expected[11]. The combination of Cellartis Cardiomyocytes, the automated patch clamp systems Patchliner and SyncroPatch 384PE, and the CardioExcyte 96 provides an excellent assay for drug safety testing in the light of the CiPA initiative.

References

1. Sager, P.T., *et al.,* 2014. Am. Heart J. 167(3): 292-300
2. Gintant, G., *et al.,* 2016. Nat. Rev. Drug Discov. 15(7):457-71
3. Li, R.A., *et al.,* 2002. Mol. Pharmacol. 61:136–141
4. Catterall, W.A., *et al.,* 2005. Pharmacol. Rev. 57:397–409
5. Bénitah, J. P., *et al.,* 1992. Pflugers Archiv. 421(2–3):176–187
6. Li, G-R., *et al.,* 1999. Am. J. Physiol. Heart. Circ. Physiol. 276:H98-H106
7. Shen, J-B., *et al.,* 2000. JPET. 294(2):562-570
8. Zhang, S., *et al.,* 1999. Circ. Res. 84:989-988
9. Zhan, S., *et al.,* 1997. J. J. Cardiovasc. Pharmacol. 30: 489-496
10. Sanguinetti, M.C. & Jurkiewicz, N.K., 1990. JGP. 96:195-215
11. Nguemo, F., et al., 2012. Cell. Physiol. Biochem. 29:819-832

Methods

Cells

Cellartis® Cardiomyocytes were kindly provided by Takara Bio (Takara Bio Europe, Cat nr. Y10075).

Cell culture

Cardiomyocytes were cultured and harvested according to Nanion's standard cell culture protocols for electrophysiology or plated directly on the NSP-96 plates for impedance/EFP measurements.

Electrophysiology

Whole cell patch clamp recordings were conducted according to Nanion's standard procedures for the Patchliner, SyncroPatch 96 and SyncroPatch 384PE.

Impedance and EFP measurements

Impedance and EFP measurements were performed according to Nanion's standard procedures for the CardioExcyte 96.

Nanion Technologies GmbH
Gabrielenstr. 9
80636 Munich, Germany

phone +49 89 218997972
fax +49 89 218997960
www.nanion.de • info@nanion.de