Qualification of Patch Ready Cells on a SyncroPatch® 384PE

Recombinant cell lines functionally expressing human cardiac ion channels are a valuable tool to test new drug entities for a potential side effect to induce proarrhythmia. It can be difficult to maintain a constant quality of these cell lines in a continually passaged culture making this process incompatible with a routine screening in high throughput mode. Here we demonstrated the preparation of patch ready cells prepared from five cell lines expressing recombinant ion channels (B'SYS, Switzerland) which are recommended by the CiPA initiative for drug safety testing. The patch ready cells have been qualified by automated patch-clamping on a SyncroPatch® 384PE (Nanion, Germany) to demonstrate their applicability in high -throughput cardiotoxicity testing.

Cell Line	Genes
CHO Kir 2.1	KCNJ2
CHO K_V4.3/KChIP2	KCND3, KCNIP2
CHO K_VLQT1/mink	KCNQ1, KCNE1
CHO hERG-DUO	KCNH2
CHO Na_V1.5-DUO	SCN5A

Tab. 1 CiPA Panel Cell Lines by B´SYS

introduction

The Comprehensive in Vitro Proarrhythmia Assay (CiPA) initiative employ analysis of a panel of cardiac ion channels known to be targeted by drugs resulting in heart failure.

The swiss CRO B'SYS generated and validated recombinant cell lines which stably express ion channels of the CiPA panel for safety pharmacology screening (Tab. 1). Optimized for this cell lines, acCELLerate developed a protocol to freeze the cells at a highly functional state. Instantly after thawing and without prior cultivation, these Patch Ready Cells (PRCs) exhibit a strong and functional expression of the ion channels and display a smooth but durable cell membrane enabling automated patch clamp in high-throughput mode.

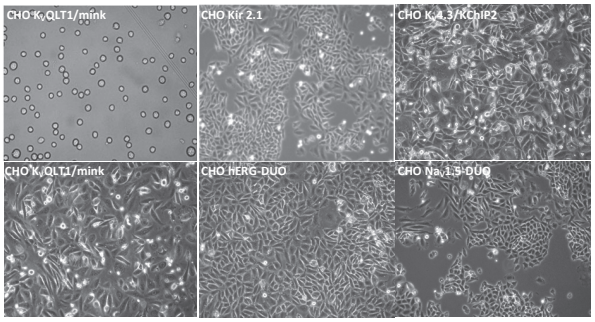

Fig. 2: Patch Ready Cells after thawing (top left) and 48h.

preparation of cells

A vial of Patch Ready Cells was quickly thawed at 37°C in a water bath: The cells were washed in 8 ml prewarmed recovery buffer and centrifuged carefully at 80xg. The lose cell pellet was resuspend in standard external solution and incubated for 30 minutes at room temperature. All cell lines recovered well from the frozen stock and displayed a high viability >90%, low amount of debris, and almost no aggregation (Fig. 1). The suspended cells display a round shape, have a smooth surface and adhere quickly within 48 hours (Fig. 2).

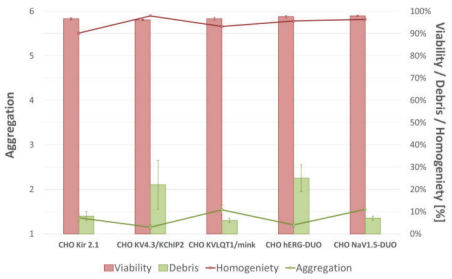

Fig. 1: Viability of Patch Ready Cells

automated patch clamp

After thawing and resuspension in external solution the Patch Ready Cells were directly tested on a SyncroPatch 384PE® (Nanion, Germany). Measurements have been acquired either from single holes or aggregated from four holes of the chip in whole cell or perforated patch mode.

After a good seal was established the ion channels were activated by individual voltage protocols which had been previously developed by Nanion. Specific ion channel blockers were added at different concentrations simultaneously to individual holes of the chip.

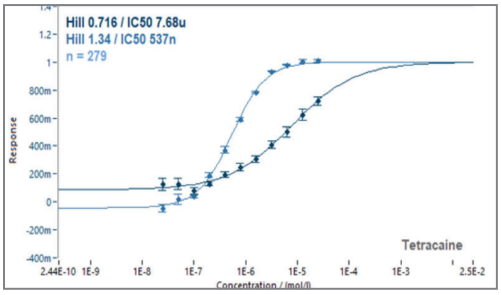

Fig. 3: NaV1.5. Peak & late currents acquired after blockage with Tetracaine.

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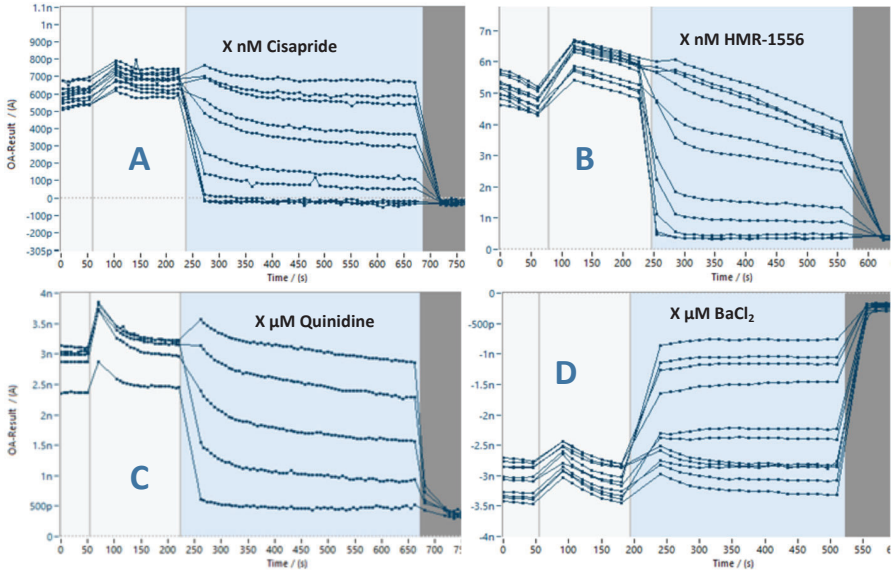

Fig. 4: Dose dependent blockage of currents acquired form hERG (A), K_V4.3-KCHip2 (B), K_VLQT7minK (C) and Kir2.1 (D) by specific inhibitors.

Na_V1.5 (Fig. 3)

The CHO-Na_V1.5 Patch Ready Cells displayed a good seal rate of 84.9 %. An average peak current of -5.2±3.4nA (n=279) was obtained. Peak and late currents could be acquired from the cells and blocked with Tetracaine ($IC_{50(peak)}$ = 7.7 µM / $IC_{50(late)}$ = 0.52 µM).

hERG (Fig. 4A)

The CHO-hERG-DUO Patch Ready Cells were measured in perforated mode to compensate a decreasing sealing resistance. Because of the low current of <100pA data from four holes of the chip were aggregated. The Patch Ready Cells displayed a good seal rate of 81.2 %. An average peak current of 0.67±0.27nA (n=285) was obtained (Fig. 4A).

K_V4.3-KCHip2 (Fig. 4B)

Measurements from the CHO-K_V4.3-KCHip2 Patch Ready Cells were acquired in perforated mode to compensate the run-down and aggregated from 4 holes of the chip. The Patch Ready Cells displayed a seal rate of 72.4 % in perforated patch mode (86.5 % in whole cell). An average peak current of 3.0±1.8nA (n=226) was obtained (Fig 4B).

K_VLQT1/minK (Fig. 4C)

Measurements from the CHO-KvLQT1/minK Patch Ready Cells were acquired in whole cell mode but aggregated from 4 holes of the chip. The Patch Ready Cells displayed a good seal rate of 82.8 %. An average current of 5.7±1.7nA (n=290) was obtained.

CHO-Kir2.1 (Fig. 4D)

CHO-Kir2.1 Patch Ready Cells were measured in whole cell mode from single hole of the chip. The Patch Ready Cells displayed a very good seal rate of 85.2 %. An average current of 3.1±0.9nA (n=238) was obtained (Fig 4D).

discussion

Cost effective screening tests need to be developed to asses adverse effect drug candidates as early as possible. One of the major bottlenecks is the sufficient and on-time supply of cells, which are classically taken from a continuously passaged maintenance culture. Patch Ready Cells can be used reliably on automated patch clamp devices designed for routine high-throughput applications. An average overall success rate of greater than 80% was obtained from all cell lines. The combination of Patch Ready Cells with the SyncroPatch® 384PE provided a versatile set-up to assess the safety pharmacology of lead substances early in the drug discovery process.

Channel	Mode	Success	Blocker / IC_{50}
Na_V1.5 (peak)	WC (1)	79.9 %	Tetracaine: 7.7 mM
Na_V1.5 (late)	WC (4)	80.5 %	Tetracaine: 0.52 mM
hERG	Perf. (4)	80.7 %	Cisapride: 31.4 nM
K_V4.3-KChip2	Perf. (4)	84.1 %	Quinidine: 36.9 mM
K_VLQT1/minK	WC (4)	82.8 %	HMR-1556: 101 nM
Kir2.1	WC (1)	81.0 %	$BaCl_2$: 5.0 mM

Tab. 2: Overall success rate of blocking experiments performed with Patch Ready Cells.

acknowledgements

- recombinant ion channel cell lines were provided by B'SYS, Switzerland.
- patch clamping experiments on the SyncroPatch® 384 PE have been performed by Nanion, Germany

related products

PRC—Patch Ready Cells (5 million cells/vial) recovery & patch buffer included

RE302	CHO Kir 2.1
RE303	CHO K_V4.3/KChIP2
RE304	CHO K_VLQT1/minK
RE305	CHO hERG-DUO
RE306	CHO Na_V1.5-DUO