Qualification of Patch Ready Cells on a SyncroPatch 384PE

Recombinant cell lines that functionally express human cardiac ion channels are a valuable tool for testing new drugs for potential side effects that 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 routine screening in high-throughput mode. Here we demonstrate 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 tested by automated patch-clamp 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 employs 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 these cell lines, acCELLerate developed a protocol to freeze the cells in 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.

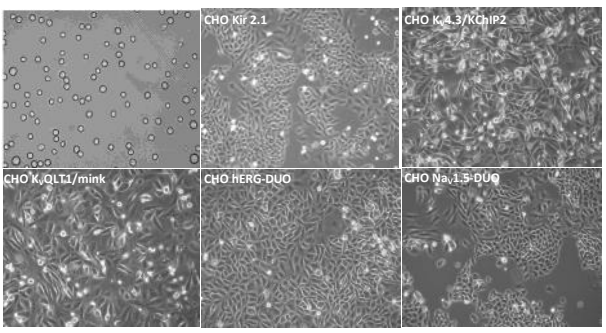

Fig. 2: Patch Ready Cells after thawing (top left) and 48h.

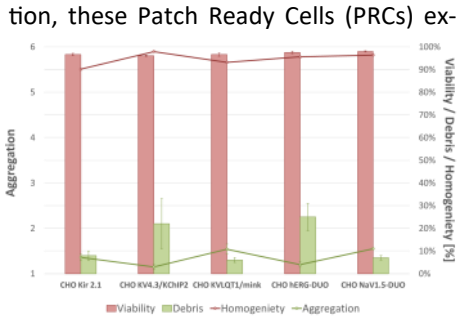

Fig. 1: Viability of Patch Ready Cells

preparation of cells

A vial of Patch Ready Cells was quickly thawed at 37°C in a water bath: The cells were washed in 8ml pre-warmed recovery buffer and centrifuged carefully at 80xg. The lose cell pellet was resuspended 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).

automated patch clamp

After thawing and resuspension in external solution the Patch Ready Cells were directly tested on a SyncroPatch 384PE (Nanion, Germany). Measurements were acquired either from single- or four-hole chips in whole cell or perforated patch clamp mode.

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

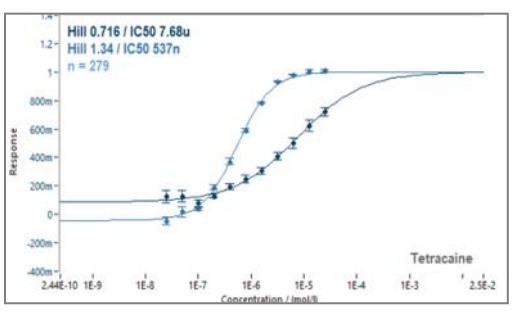

Fig. 3: **Na_V1.5-DUO** Peak & late currents acquired after blockage with Tetracaine.

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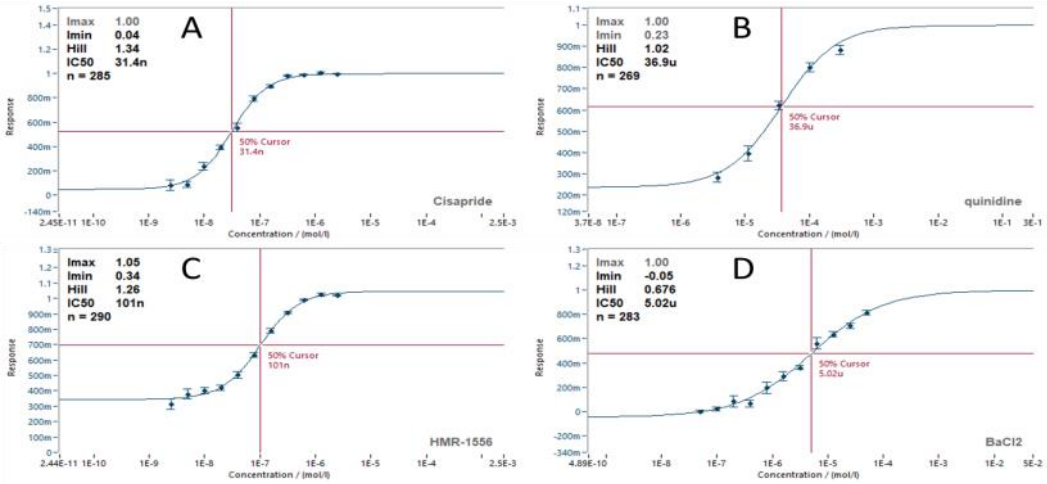

Fig. 4: Concentration dependent blockage of currents acquired form hERG-DUO(A), K_V4.3-KChip2 (B), K_VLQT7minK (C) and Kir2.1 (D) by specific inhibitors.

Na_V1.5-DUO(Fig. 3)

The CHO-Na_V1.5 Patch Ready Cells displayed a good seal rate of 84.9% >500MOhm. 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 ($IC_{50(peak)}$ = 7.7µM / $IC_{50(late)}$ = 0.52µM).

hERG-DUO(Fig. 4A)

The CHO-hERG-DUO Patch Ready Cells were measured in perforated patch mode to prevent current rundown. Because of the low average current amplitude of <100pA per cell, the cells were recorded on 4-hole chips where the sum of the currents of all four cells in each well is used. The Patch Ready Cells displayed a good seal rate of 81.2% >100MOhm. An average peak current of 0.67±0.27nA (n=285) was obtained.

K_V4.3-KChip2 (Fig. 4B)

Measurements from the CHO-K_V4.3-KChip2 Patch Ready Cells were acquired in whole cell mode using 4-hole chips. The Patch Ready Cells displayed a seal rate of 86.5% >100MOhm. An average peak current of 2.5±1.5nA (n=269) was obtained.

K_VLQT1/minK (Fig. 4C)

Measurements from the CHO-KvLQT1/minK Patch Ready Cells were acquired in perforated mode using 4-hole chips. The Patch Ready Cells displayed a good seal rate of 82.8% >100MOhm. 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 using single hole chips. The Patch Ready Cells displayed a very good seal rate of 85.2% >500MOhm. An average current of 3.1±0.9nA (n=238) was obtained (Fig 4D).

discussion

Cost effective screening tests must be developed to assess adverse effects of 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 >80% was obtained from all cell lines. The combination of Patch Ready Cells with the SyncroPatch 384PE provide 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: 7.7µM
Na_V1.5 (late)	WC (1)	80.5 %	Tetracaine: 0.52µM
hERG-DUO	Perf. (4)	80.7 %	Cisapride: 31.4nM
K_V4.3-KChip2	WC (4)	84.1 %	Quinidine: 36.9µM
K_VLQT1/minK	WC (4)	82.8 %	HMR-1556: 101nM
Kir2.1	WC (1)	81.0 %	$BaCl_2$: 5.0µM

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 were performed by Nanion Technologies, 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/KChip
RE304	CHO K_VLQT1/minK
RE305	CHO hERG-DUO
RE306	CHO Na_V1.5-DUO