Patch Ready Cells – Flexible Tools in Cardiotoxicity Testing

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Introduction

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. 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. 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.

Here we demonstrate the preparation of Patch Ready Cells derived from six cell lines expressing recombinant cardiac ion channels. The cell lines were generated and validated by B'SYS as well as Steinbeis Innovationszentrum Zellkulturtechnik together with NMI TT Pharmaservice using their proprietary IGAMI® technology. 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.

Preparation of Patch Ready 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 100xg. The cell lines were expanded in T-flask and CellSTACKs (Corning) to a batch of about 2 billion cells and maintained at a sub-confluent cell density of less than 20,000 cells/cm². The cells were harvested with Accutase and resuspended in freezing medium containing 5% DMSO for cryoprotection. The suspension was automatically dispensed into cryovials at a cell density of 10 million cells per vial using an XSD-Biofill. Cryopreservation was performed in a Cryomed 7452 controlled rate freezer using an optimized freezing protocol. Vials, thawed for quality control displayed a high viability >90%, low amount of debris and almost no aggregation (Fig. 1). The suspended cells displayed a round shape and a smooth surface directly after thawing (Fig. 2).

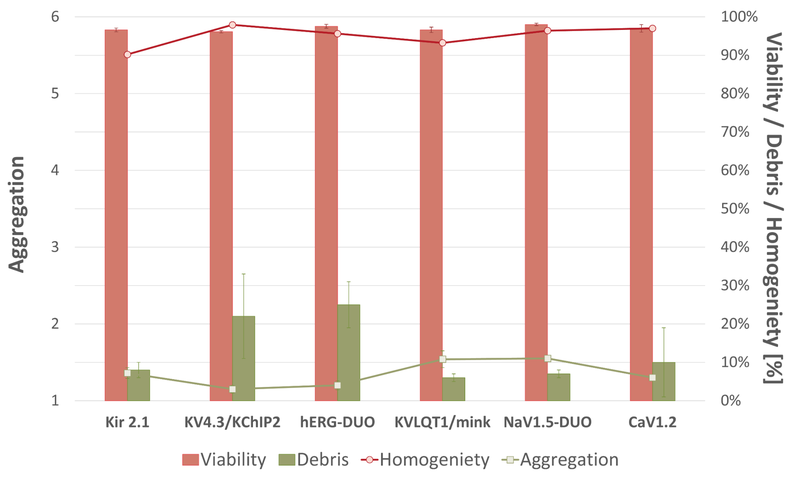

Fig. 1: Cell culture parameter of Patch Ready Cells directly after thawing

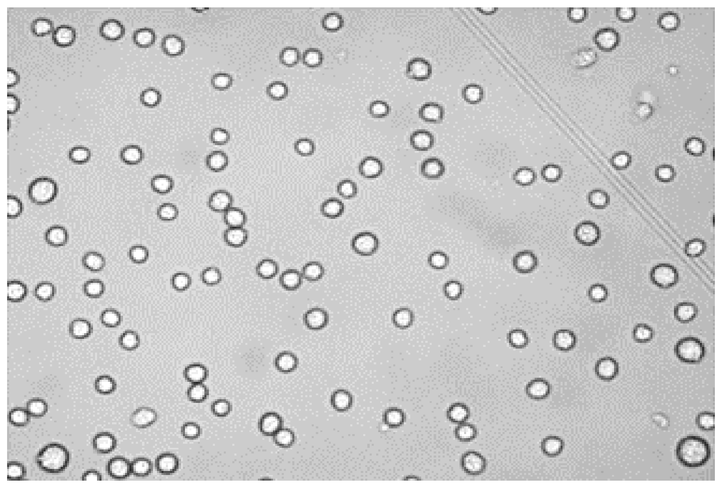

Fig. 2: Suspended Patch Ready Cells directly after thawing

High Throughput Testing using Automated Patch Clamp on a SyncroPatch 384PE

After thawing and resuspension in an 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. 3-8).

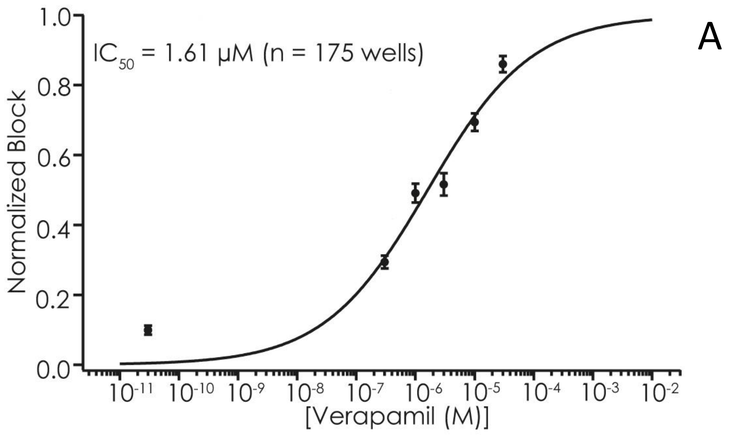

Fig. 3: CHO-$Ca_V1.2$ (A) Measurement was performed in perforated patch mode using 4-hole chips. The cells displayed a seal rate of 94% >100MΩ and an overall success rate of 75%. The current was blocked with Verapamil, obtaining an IC_{50} of 1.61 µM. **(B)** Over a measurement period of >10 min, hardly any rundown (<10%), which is common for voltage-gated calcium channels, could be detected.

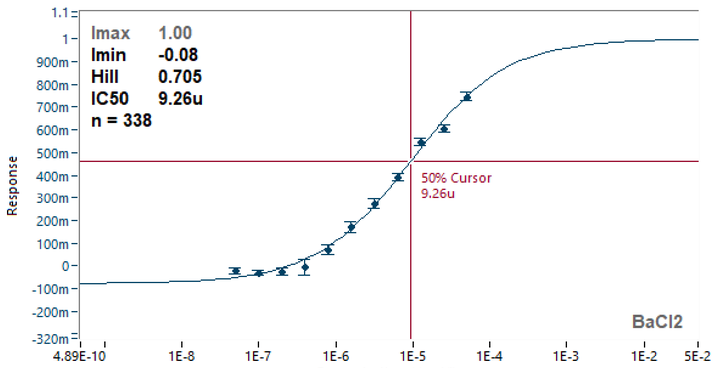

Fig. 4: CHO-Kir2.1 Patch Ready Cells were measured in whole cell mode using single hole chips. The Patch Ready Cells displayed a seal rate of 85.2% >500MΩ and an overall success rate of 81%. The channel was blocked with $BaCl_2$ obtaining an IC_{50} of 5.0µM.

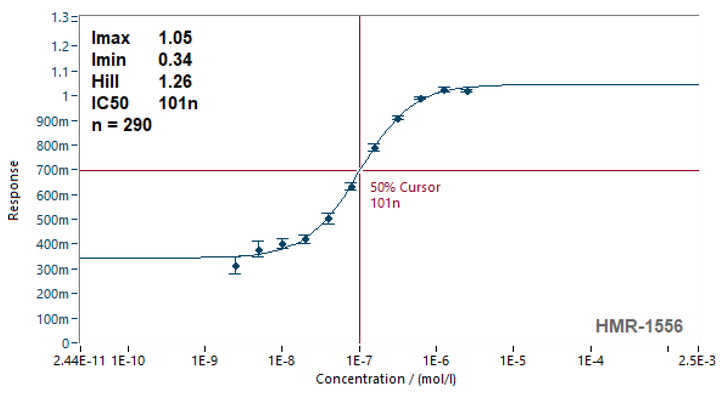

Fig. 6: CHO-KvLQT1/mink The cells were measured in perforated patch mode using 4-hole chips. The cells displayed a seal rate of 82.8% >100MΩ and an overall success rate of 82.8%. The current was blocked with HMR-1556 obtaining an IC_{50} of 101nM.

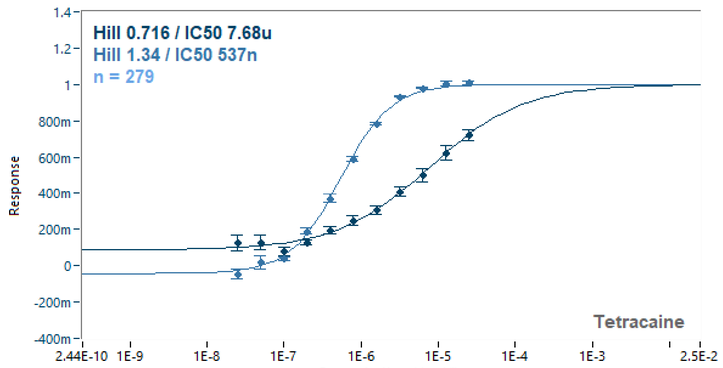

Fig. 5: CHO-$Na_V1.5$-DUO Measurement was performed in whole cell mode using single hole chips. The cells displayed a seal rate of 84.9% >500MΩ and an overall success rate of 79.9% (peak) and 80.5% (late). Peak and late currents could be acquired from the cells and blocked with Tetracaine obtaining an $IC_{50(peak)}$ of 7.7µM (dark blue) and $IC_{50(late)}$ of 0.52µM (light blue).

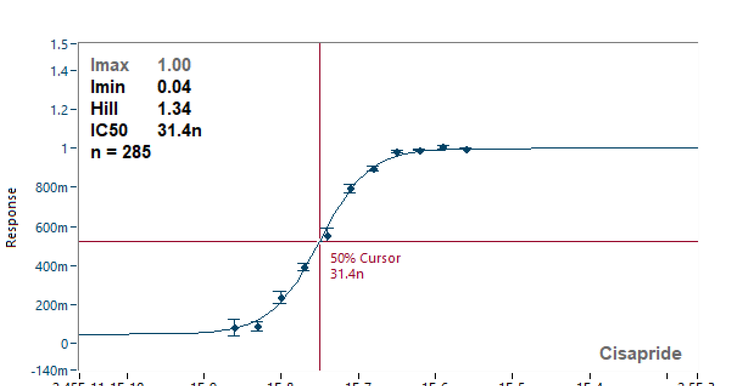

Fig. 7: CHO-hERG-DUO Measurement was performed in perforated patch mode using 4-hole chips. The cells had a seal rate of 81.2% >100MΩ and an overall success rate of 80.7%. The current was blocked with Cisparide obtaining an IC_{50} of 31.4nM.

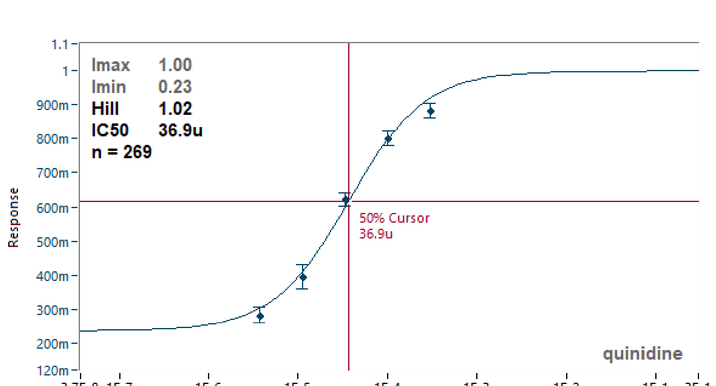

Fig. 8: CHO-$K_V4.3$-KChip2 Measurement was performed in whole cell mode using 4-hole chips. The cells displayed a seal rate of 86.5% >100MΩ and an overall success rate of 84.1%. The current was blocked with Quinidine obtaining an IC_{50} of 36.9µM.

Summary

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 adequate 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. High average overall success rates have been obtained from all cell lines. The combination of Patch Ready Cells with the SyncroPatch 384 PE provides a versatile set-up to assess the safety pharmacology of lead substances early in the drug discovery process.

Since Patch Ready Cells can be produced in large homogenous batches and stored in liquid nitrogen for a long time, the same prequalified cells can be used for repeated patch clamp experiments, or even in different testing labs around the world which exceptional increases the reproducibility from assay-to-assay and from lab-to-lab.

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