Investigating the effect of temperature and trafficking modulators on hERG current using high throughput automated patch

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1 Introduction

The human Ether-à-go-go Related Gene (hERG) channel is a potassium channel essential for cardiac repolarization. Disruptions to hERG function, caused by mutations or drug interactions, can result in long QT syndrome and life-threatening arrhythmias[1]. Consequently, evaluating drug effects on the hERG current is a key component of cardiac safety testing, predominantly performed using automated patch clamp (APC) systems.

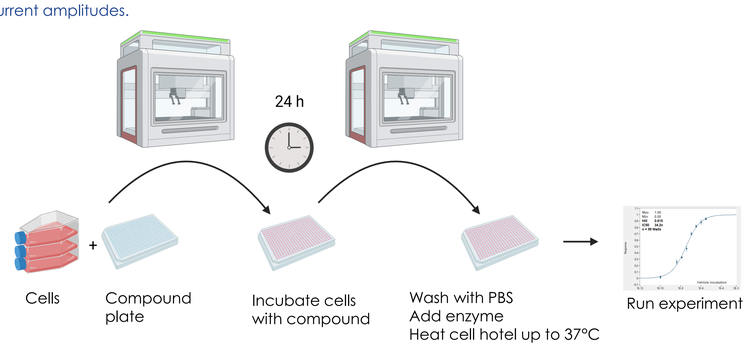
While hERG safety studies typically focus on rapid direct drug-channel interactions, some compounds impair hERG functionality by altering the channel's surface density through trafficking modulation. These compounds act more slowly, requiring overnight incubation to allow trafficking changes to occur before assaying the cells the next day.

In this study, we employed **automated cell harvesting** integrated with automated patch clamp (APC; SyncroPatch 384) to investigate the effects of culture conditions and trafficking-modulating compounds on hERG current. Specifically, we assessed temperature modulation[2,3] and incubation with reference compounds like nocodazole, a cell division inhibitor[4,5], and bepridil, a known hERG blocker with trafficking effects[6]. This automated approach enabled high throughput and consistent preparation of cells directly from 384-well plates, ensuring robust and reproducible results. By integrating APC with automated cell preparation, this study highlights a scalable and efficient strategy for cardiac safety research, offering valuable insights into the modulation of hERG current under varied conditions.

2 Methods

Cell culture

We used HEK293 cells expressing hERG (kindly provided by SB Drug Discovery). The cells were harvested and transferred to a µCLEAR compound plate that is optimized for cell culture (Greiner #781091), prefilled with test compounds using the SyncroPatch384 liquid handler and placed in an incubator overnight. On the day of experiment the cells are washed with PBS and incubated with TrpLe for 15 min on the "cell hotel" that is heated to 37 °C, again using the SyncroPatch 384. The cells are separated with fast mixing steps and are then transferred to the NPC-38 chip for recording hERG current amplitudes.



3 Optimizing assay conditions

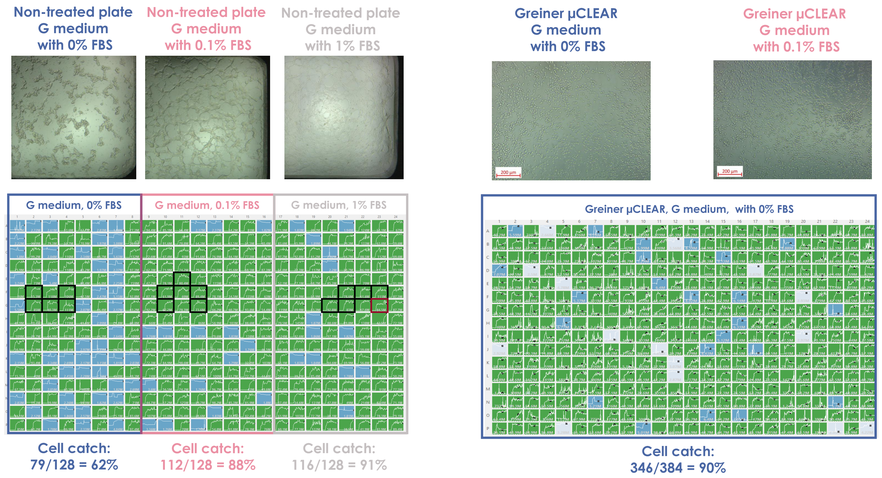
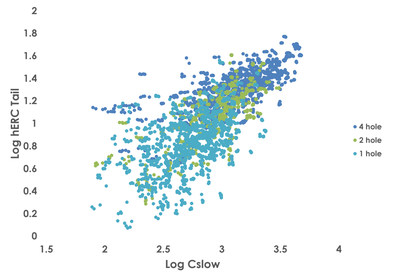


Figure 1: Effect of serum on cell morphology and cell catch. Cell morphology and cell catch were tested as a function of different concentrations of serum in non-coated and coated compound plates. The cell catch onto the patch clamp hole was also affected negatively by the absence of serum when using non-coated plates. Since serum can affect small molecule compounds, we reduced the final concentration of serum to between 0.1% and 1% FBS in untreated compound plates. Incubation with 0% FBS in µCLEAR compound plates resulted in improved morphology and cell catch.



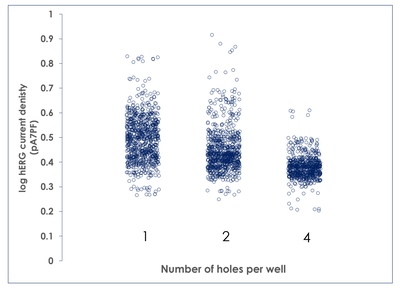
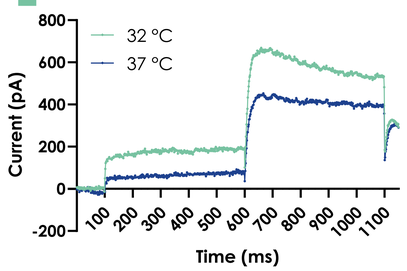


Figure 2: Determination of optimal chip and analysis parameters. A chip designed for assay development with an array of 1, 2 or 4 holes per well was used to find the optimal number of holes per well. hERG tail current increased as a function of Cslow – a current density parameter is the best choice.

4 holes reduces the variability of the tail current between wells optimally. CV of log Tail hERG tail current was determined to be 18%, 19% and 12% with 1, 2 and 4 holes respectively.

4 Temperature and compounds



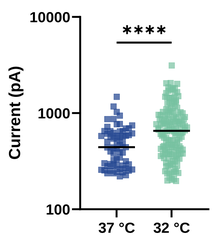
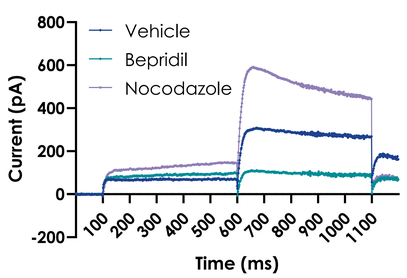


Figure 3: Effect of incubation at lower overnight temperature. Example raw hERG traces from 32 °C and 37 °C and scatter plot of hERG tail currents from individual wells. Cells were incubated at two temperatures overnight and the responses were tested the next day using 1-hole plates. A significant increase in hERG tail current of 64% was found between the two conditions.



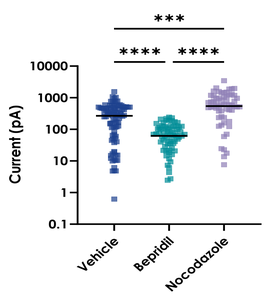


Figure 4: Effect of overnight incubation of trafficking compounds. Example raw hERG traces and scatter plot of hERG tail currents from individual wells from vehicle control, bepridil and nocodazole test conditions.

Overnight incubation in a 384 well µCLEAR compound plate with 30 µM of bepridil or 25 µM nocodazole was assayed the following day and resulted in a significant 75% reduction of hERG tail current by bepridil and a significant increase in hERG tail currents by 100% by nocodazole. We did not find any effects of 0.1% FBS being present during compound incubation.

5 Conclusions

- We have developed a method that makes overnight compound incubation possible in a practical and automated manner in a 384 well format.
- This method allows for large scale testing of compound effects on trafficking.
- Reduced incubation temperature has a positive effect on expression of hERG in the membrane.
- Bepridil has an inhibitory effect of hERG trafficking and nocodazole increases hERG expression in the membrane.
- The method can be applied to other targets where trafficking is a key parameter like the CFTR channel.

References

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