There is no F in APC: Reliable fluoride-free recordings on the SyncroPatch 384

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Cells were kindly supplied by SB Drug Discovery or Charles River.
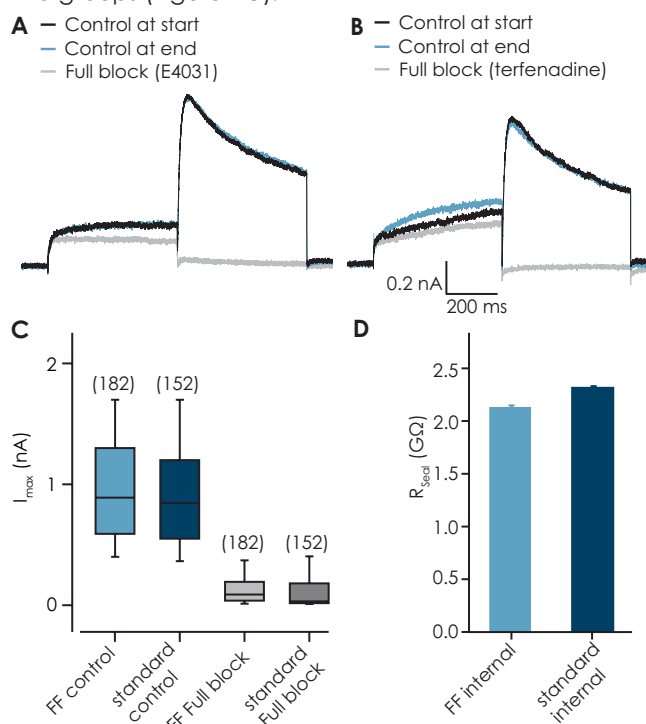
charles river   sb drug discovery

Summary

Automated patch clamp (APC) instruments are used for a wide variety of applications ranging from basic research into channelopathies and biophysical characteristics of ion channels, through to routine cardiac safety testing. Their use in cardiac safety screening has increased over the years and APC is now an established and accepted technique in most, if not all, safety testing laboratories. It is well known that fluoride is often used in the internal solution in APC experiments to improve the seal resistance. The presence of external calcium (or other divalent cation) further improves the seal by a mechanism thought to be due to the formation of CaF_2 crystals at the interface between the pipette or micro-pore and the cell as described in a recent patent application[1]. Even in manual patch clamp experiments, fluoride has been used to record voltage-gated Na^+ channels for over 20 years[2,3,4], despite known effects on voltage dependence of conductance, and steady-state fast inactivation[4-6] and its inhibition of protein phosphatase[7]. Fluoride is used because it improves the seal and allows stable measurements to be performed over long periods of time[8]. However, because there are some experiments where it is advantageous to use physiological, fluoride-free internal solutions and external solution that does not use divalent 'seal enhancer' solutions, we have developed a method that allows fluoride-free, physiological solutions to be used with good success rates. We demonstrate this using the cardiac ion channels hERG expressed in HEK293 cells (SB Drug Discovery) and $Na_V1.5$ expressed in CHO cells (Charles River).

Results

hERG expressed in HEK293 cells were recorded on the SyncroPatch 384 using fluoride-free (FF) internal solution (Figure 1A) or standard (fluoride-containing) internal solution (Figure 1B). Little difference in current amplitude (Figure 1C) and R_{Seal} (Figure 1D) was observed between the two groups (Figure 1C).

A — Control at start — Control at end — Full block (E4031)

B — Control at start — Control at end — Full block (terfenadine)

0.2 nA 200 ms

C I_{max} (nA)

(182) (152)

(182) (152)

FF control, standard control, FF Full block, standard Full block

**D** R_{Seal} (GΩ)

FF internal, standard internal

Figure 1: hERG-mediated currents recorded on the SyncroPatch 384. **A** hERG currents from an example cell recorded using FF internal solution. **B** hERG currents recorded from an example cell using standard internal solution. **C** Box plot of current amplitudes for an example NPC-384 chip in FF and standard internal solution. The distribution of current amplitudes is not statistically significantly different (P=0.7, unpaired Student's t test). The current amplitude after block with E4031 or terfenadine is also shown. **D** Bar graph of R_{Seal} values for one experiment for 182 and 152 wells for FF and standard internal, respectively. Shown are mean ± S.E.M.

Current amplitude was also stable over time, the current after 5 min was not statistically different from the current at the start of the experiment in either FF or standard internal. The hERG current was completely inhibited by a full block concentration of either E4031 or terfenadine (Figure 1).

Success rates (% available wells) were calculated for multiple NPC-384 chips using either standard internal solution or FF internal solution and no use of 'seal enhancer'. Although success rates were better when fluoride was used, as expected, success rates of 34 ± 8% (n = 32 chips) were achieved without fluoride for wells with $R_{Seal} \geq 1G\Omega$ at the end of the experiment, and this increased to 57 ± 9% if the quality control parameter $R_{Seal} \geq 0.25\ G\Omega$ at the end of the experiment was used, giving a success rate of almost 60% for completed experiments. There was little loss of wells over time, the success rate at the end of the experiment was similar to that at the start (Figure 2).
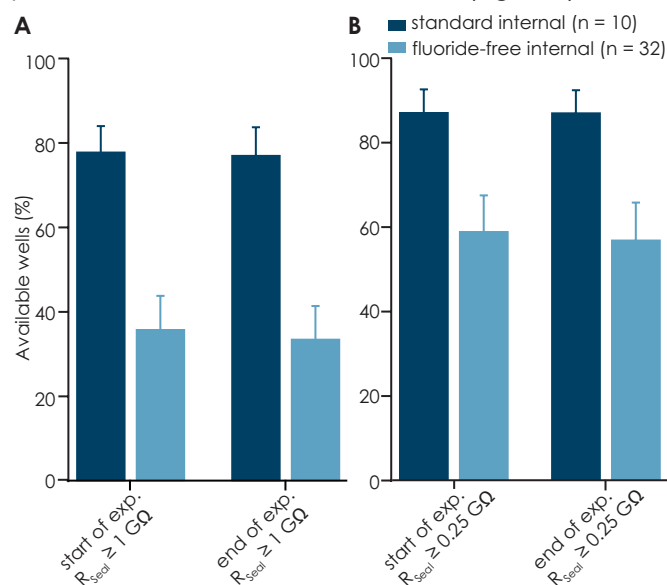

Figure 2: Bar chart of success rates for experiments using standard internal solution and FF solution for HEK293 cells expressing hERG. A Bar chart using QC parameter $R_{Seal} \geq 1\ G\Omega$ at the start and end of the experiment. **B** Bar chart using QC parameter $R_{Seal} \geq 0.25\ G\Omega$ (acceptable R_{Seal} for screening) at the start and end of the experiment. Shown are mean ± S.D. values for 10 (standard internal) and 32 (FF) NPC-384 chips.

We also used CHO cells expressing the cardiac $Na_V1.5$ channel. Using these cells, success rates for $R_{Seal} \geq 1\ G\Omega$ were 42 ± 9% in FF versus 78 ± 5% in standard solution (Figure 3A) were achieved. This increased to a success rate of 55 ± 12% for FF if the quality control parameter $R_{Seal} \geq 0.25\ G\Omega$ was used and 86 ± 6% for standard solution (Figure 4B), a cutoff which indicates an acceptable R_{Seal} for screening. There was little loss of wells over time, the success rate at the end of the experiment was similar as at the start.
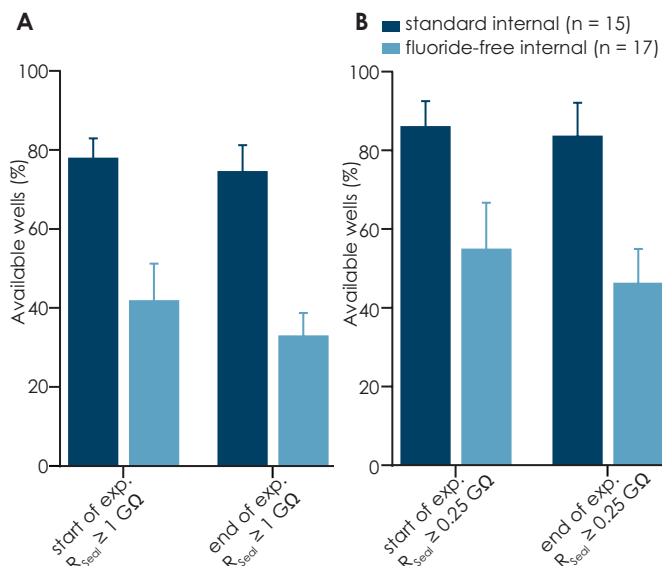
Figure 3: Bar chart of success rates for experiments using standard internal solution and FF solution for CHO cells expressing $Na_V1.5$. A Bar chart using QC parameter $R_{Seal} \geq 1\ G\Omega$ at the start and end of the experiment. **B** Bar chart using QC parameter $R_{Seal} \geq 0.25\ G\Omega$ (usable wells) at the start and end of the experiment. Shown are mean ± S.D. values for 10 (standard internal) and 32 (FF) NPC-384 chips.
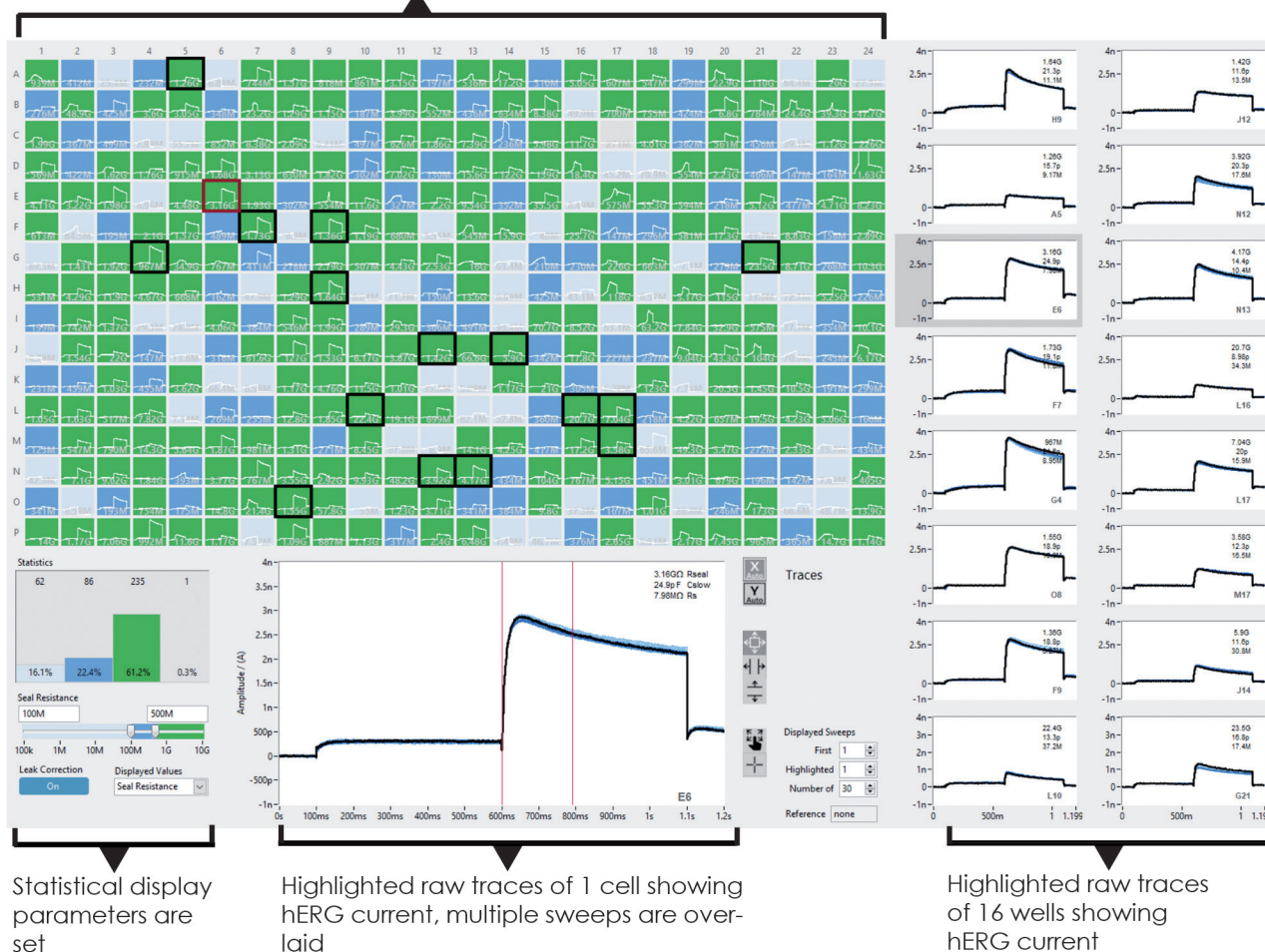
$Na_V1.5$-mediated currents were stable over time in both FF physiological internal solution or standard internal solution (Figure 4). Peak amplitudes were, however, larger in FF internal solution compared with standard internal solution (p<0.01, unpaired Student's t test; Figure 3C). $Na_V1.5$ was completely inhibited by tetracaine (full block).
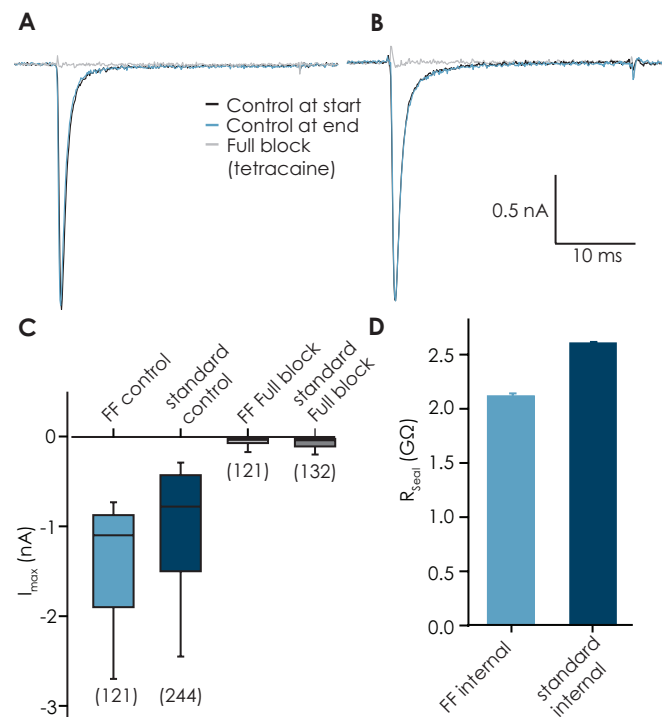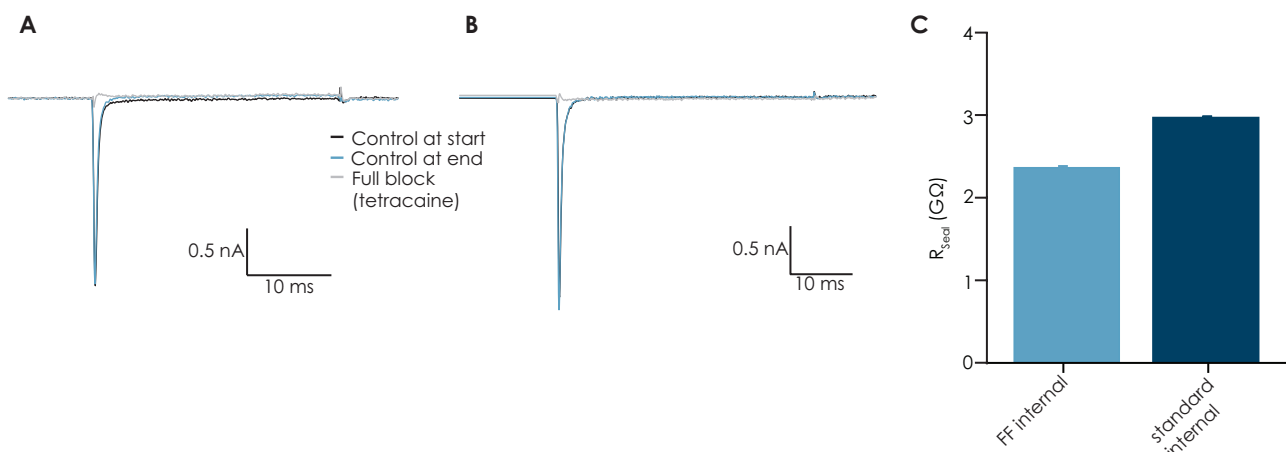

Figure 4: $Na_V1.5$-mediated currents recorded on the SyncroPatch 384. A $Na_V1.5$ currents recorded from an example cell using FF internal solution. **B** $Na_V1.5$ currents recorded from an example cell using standard internal solution. **C** Box plot of current amplitudes for an example NPC-384 plate in FF and standard internal solution. The current amplitude after block with tetracaine is also shown. **D** Bar graph of R_{Seal} values for one experiment for 121 and 244 wells for FF and standard internal, respectively. Shown are mean ± S.E.M.

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384 color coded depictions of data traces eases judgement of success rate

Statistical display parameters are set

Highlighted raw traces of 1 cell showing hERG current, multiple sweeps are over-laid

Highlighted raw traces of 16 wells showing hERG current

Figure 5: Graphical user interface of the screening and data analysis software used on the SyncroPatch 384. Screenshot of depiction of raw current traces of hERG expressed in HEK293 cells using FF physiological internal solution. Three hundred and eighty-four small color-coded pictures as seen in the upper left part display 384 recordings. Wells are color-coordinated based on R_{Seal} where green wells have R_{Seal} > 500 MΩ, blue wells have R_{Seal} between 100 and 500 MΩ and light-blue or grey wells are < 100 MΩ or disabled. In this case, over 61% of wells had R_{Seal} > 500 MΩ.

Figure 6: Na$_v$1.5 at physiological temperature in FF and standard internal. A Na$_v$1.5 currents recorded from an example cell using FF internal solution at physiological temperature **B** Na$_v$1.5 currents recorded from an example cell using standard internal solution at physiological temperature. **C** Bar graph of R_{Seal} values for one experiment for 194 (FF) and 357 (standard) wells. Shown are mean ± S.E.M.

Figure 5 shows a screenshot of the SyncroPatch 384 software during an experiment. A color-coded over-view (based on seal resistance in this case) of all 384 wells gives the user a good impression of the success rate of the experiment. The user can choose whether to visualize raw traces or online analysis. In this case, raw current traces are shown. Over 60% of the wells had $R_{Seal} > 500$ MΩ and, therefore, wells which were used for analysis, in FF physiological internal solution. With our new FF application, the user has the opportunity to run FF physiological solution with no external seal enhancer. Alternatively, internal fluoride can be used to achieve success rates >80% for com-pleted experiments with our standard approach. Fur-thermore, we performed recordings with $Na_V1.5$ at physi-ological temperature (36 ± 1°C) where we achieved 36 ± 9% (n=17; $R_{Seal} > 0.25$ GΩ) at the end of the experi-ment without fluoride versus 89 +/-5 % (n=15; $R_{Seal} > 0.25$ GΩ) at the end of the experiment when internal fluoride was used. The mean R_{Seal} was 2.37 ± 0.01 GΩ (n = 194 wells) in FF and 2.98 ± 0.01 GΩ (n = 357 wells) in standard internal (Figure 6). $Na_V1.5$-mediated currents were also stable over time (Figure 6).

The SyncroPatch 384 is a high throughput and highly reliable automated patch clamp device which can be used with standard (fluoride-containing) internal solution for high success rates and long-lasting recordings. Alternatively, fluoride-free physiological internal solution (and no external seal enhancer) can be used with good success rates, making it possible to perform high throughput APC recordings in physiological solutions used in manual patch clamp. Taking advan-tage of the 32-well mode of the SyncroPatch 384, where part of the chip can be used at a time and the remainder of the chip can be used at a later timepoint, increases flexibility of the system making it an ideal system not only for high throughput screening of large compound libraries or safety testing, but also for small screening projects and assay development.

References

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Methods

Cells
HEK293 cells expressing hERG were kindly supplied by SB Drug Discovery. CHO cells expressing $Na_V1.5$ (Cat: CT6007) were kindly supplied by Charles River Laboratories.

Cell culture
Cells were cultured and harvested according to Nanion's standard cell culture protocols.

Consumables
For Fluoride-Free recordings the recently developed NPC-384FF chip was used, whereas for recordings with internal fluoride, the standard NPC-384 chips were used.

Electrophysiology
Whole-cell patch clamp recordings were conducted according to Nanion's standard procedure for the SyncroPatch 384. hERG was recorded using a double step voltage protocol from a holding potential of -80 mV to +60 mV for 500 ms followed by a step to -40 mV for 500 ms repeated every 15 s. Peak amplitude was measured at the start of the second voltage step. $Na_V1.5$ was measured using a single step protocol from -100 mV to -10 mV repeated every 10 s. Standard internal solution contained 110 mM KF, fluoride-free internal solution contained 110 mM K-gluconate. External solution contained 60 mM NMDG. Please ask for more details.

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