Automated Patch Clamp System Introducing Simulated I_{K1} Into stem cell-derived Cardiomycoytes Using Dynamic Clamp

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Introduction

Dynamic clamp is a powerful tool to inject real-time simulated currents into patch clamped cells[1]. This has been shown using conventional patch clamp whereby the inward rectifier current I_{K1} was introduced into human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs)[2,3]. I_{K1} is typically expressed at low levels in these cells[4], hence their membrane potential is more depolarized than that of primary cardiomyocytes[4,5], limiting their use in safety pharmacology. Introducing simulated I_{K1} into hiPSC-CMs may render them a viable alternative to scarcely available adult human cardiomyocytes.

In this study, we combined dynamic clamp with an automated patch clamp (APC) system to demonstrate that I_{K1} conductance can be added to hiPSC-CMs on this platform, while at the same time, applying automatic R_{seal} compensation (SC)[6,7]. Our results show that virtual I_{K1} can be successfully injected into hiPSC-CMs in up to 8 cells simultaneously and that R_{seal} is correctly compensated avoiding overcompensation. Our approach results in more stable resting membrane potentials and action potential duration (APD) values. Action potential (AP) shape is also improved. „L"-Type calcium channel opener BayK 8644 and channel blocker nifedipine were also tested. In addition, we compared the shape of APs at physiological versus ambient temperature.
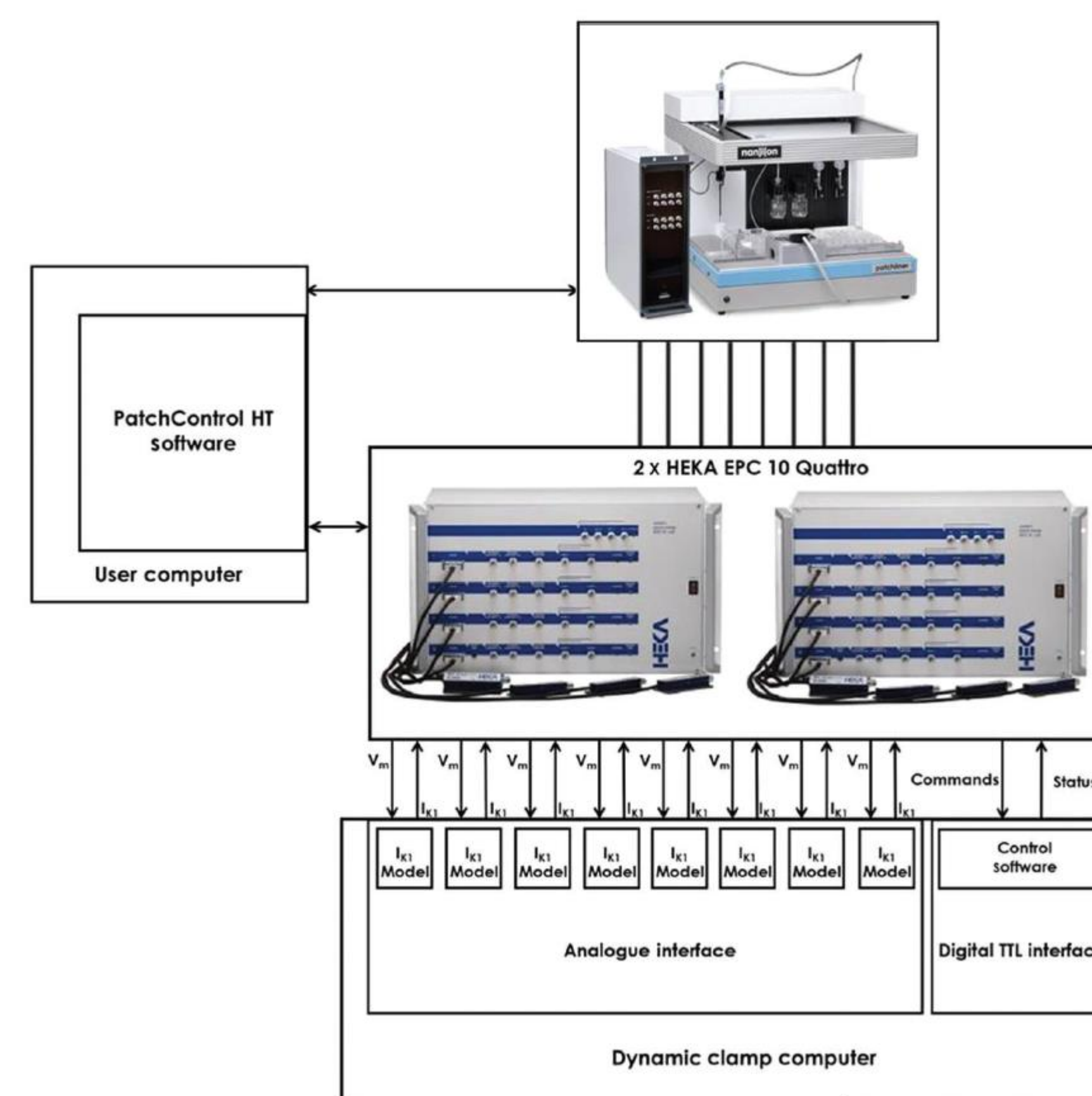
Combining dynamic clamp with APC

Figure 1 Block diagram showing connections between Patchliner, patch clamp amplifier and dynamic clamp system. Reproduced from Ref. 7.

Theory of Seal Resistance compensation

$$V_M = V_{extra} - V_{intra}$$

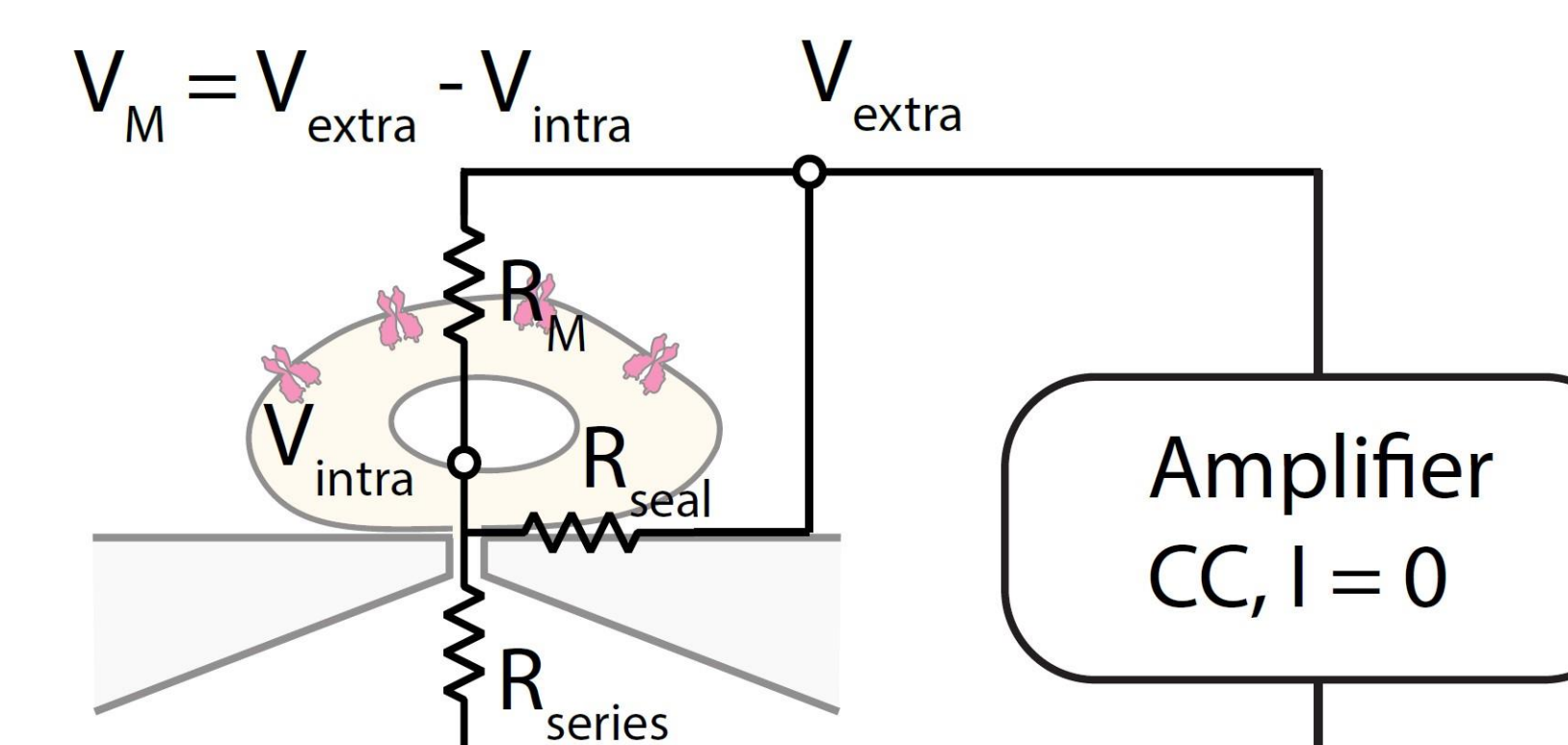**Figure 2** In current clamp mode (CC), VM can dissipate over membrane resistance (RM) or seal resistance (Rseal). By injecting VM x Rseal-1, leak through Rseal can be compensated for.

Effect of seal compensation and I_{K1} in the shape of hiPSC-CM

A Recorded action potentials
- AP with holding current only
- AP with 70% seal compensation
- AP with 70% seal compensation and 700 pS/pF I_{K1}

B Injected seal compensation
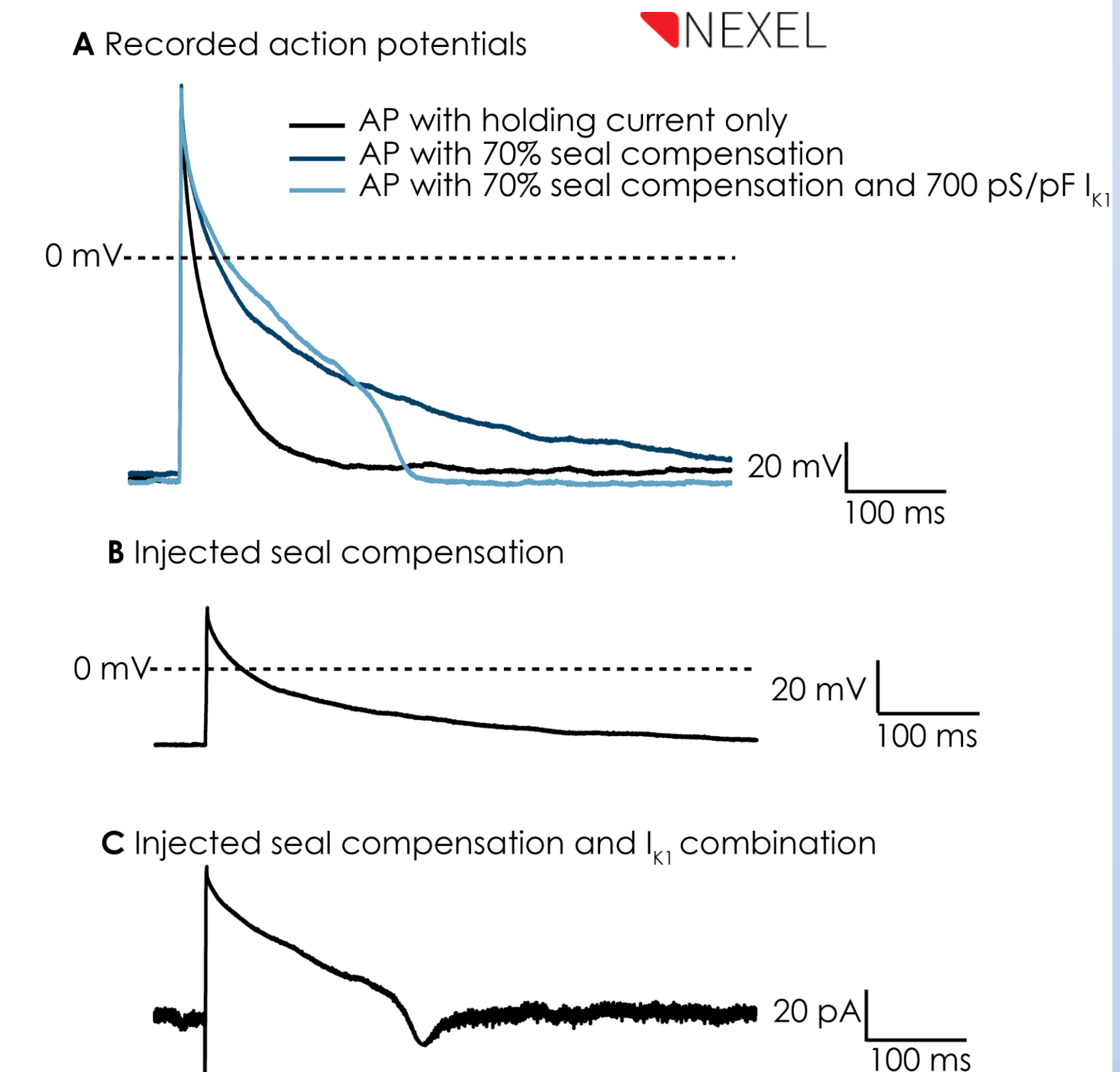
C Injected seal compensation and I_{K1} combination

Figure 3 A: Example of an action potential (AP) recorded from a Cardiosight-S® cell (Nexel) when only holding current is injected (black), when 70% seal compensation is injected (dark blue), and when 70% seal compensation and I_{K1} is injected (light blue). **B:** Injected seal compensation for the cell shown in A. **C:** Injected seal compensation and I_{K1} for the cell shown in A. B and C are shown on the same scale (data from Ref. 7).

Effect of applying seal compensation and simulated I_{K1} to hiPSC-cardiomyocytes on AP shape and pharmacology

A
- Control
- 10 µM BayK 8644
- 10 µM Nifedipine

B
C

D
BayK 8644
EC_{50} = 11.9 nM (n = 4)
- Control
- 1 nM BayK 8644
- 10 nM BayK 8644
- 100 nM BayK 8644
- 1 µM BayK 8644

E
F
- Room temperature
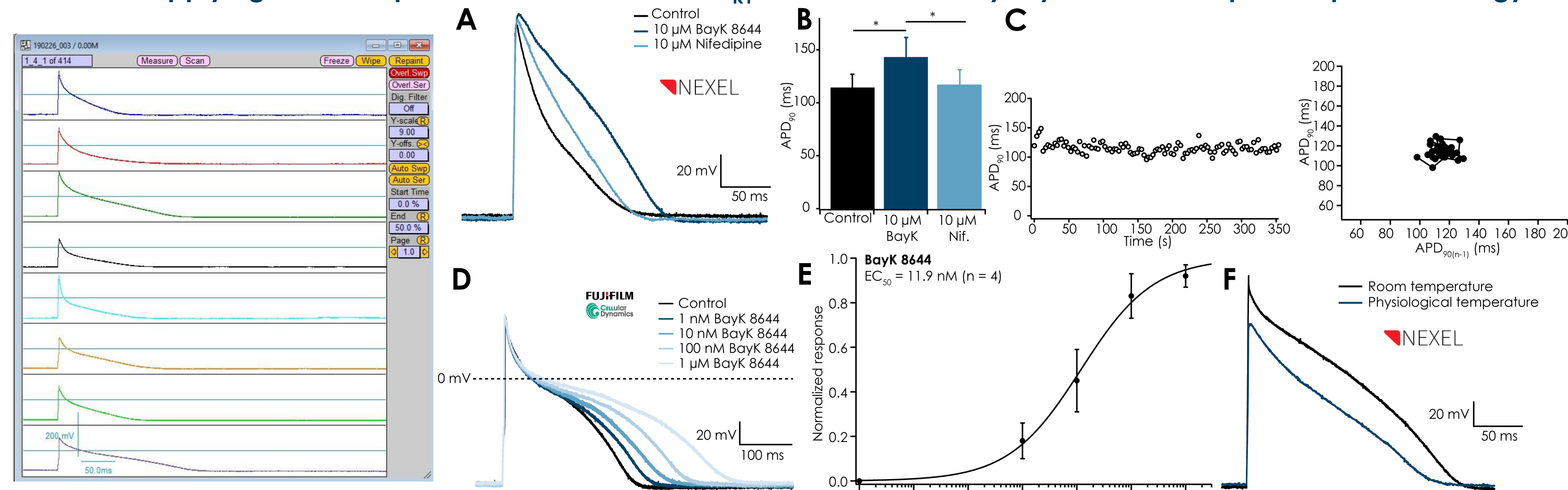- Physiological temperature

Figure 4 Screenshot taken from the PatchMaster software with 8 APs recorded in parallel from hiPSC-cardiomyocytes on the Patchliner Octo. Seal compensation combined with simulated I_{K1} using dynamic clamp resulted in longer APs.

Figure 5 A: APs recorded from Cardiosight–S® hiPSC-CMs in the presence of BayK 8644 and nifedipine. **B:** Effect of 10 µM BayK 8644 and nifedipine on APD_{90} in Cardiosight-S® hiPSC-CMs (*P<0.05, paired Student's T-test). **C:** Time course (left) and short term variability of APD_{90} recorded from Cardiosight-S®. **D:** APs recorded from iCell Cardiomyocyte² hiPSC-CMs (Fujifilm Cellular Dynamics, Inc.) under control conditions and after addition of 10 µM BayK 8644 and 10 µM nifedipine. **E:** Dose-response curve of BayK 8644 on APD_{90} in iCell Cardiomyocyte². **F:** Effect of physiological temperature on the shape of the AP (Data from Ref 7).

Conclusions

- I_{K1} and seal compensation were successfully applied to hiPSC-CMs in multiple cells simultaneously and effects on RMP and AP duration.

- Combined with the Patchliner Octo, Dynamite[8] enabled the recording of record stable APs from hiPSC-CM at a higher throughput.

- Ca^{2+} channel activator, BayK 8644, and blocker, nifedipine, prolonged and shortened AP duration, respectively, as expected and a complete concentration-response curve was recorded on an individual cell.

- APs at physiological temperature were also measured.

- Future goals:
  - Test effects of the set of drugs defined by CiPA on action potentials using dynamic clamp.
  - Upgrade technique to higher throughput devices, e.g. SyncroPatch 384i.

References
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