Contractility-based pharmacological characterization of hiPSC-derived atrial and ventricular cardiomyocytes assessed on the FLEXcyte 96

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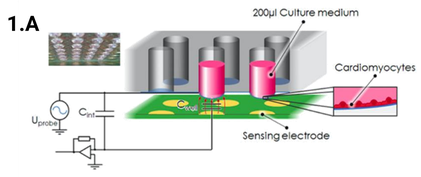
Abstract

- Commercial human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) became an important tool for preclinical cardiac risk assessment, owing to their human origin and unlimited reproducibility.
- To address chamber-related cardiac diseases like atrial fibrillation reliably, cardiac subtype-specific commercial cell lines are needed.
- Here, we compare commercially available ventricular and atrial cardiomyocytes (axoCells™, Axol Biosciences) regarding their contractile properties using FLEXcyte technology.
- General beat shape and compound-induced effects on contractile properties including beat rate, amplitude and duration were assessed on acute basis with S-Bay K8644 at 5 different concentrations ranging from 100 nM – 1 µM as well as 4-AP and carbachol with 5 concentrations ranging from 1 µM – 100 µM.
- The data shows that hiPSC-derived atrial and ventricular cardiomyocytes reproduced the different contractile phenotypes and pharmacological responses of primary cardiomyocyte sub-types suitably.

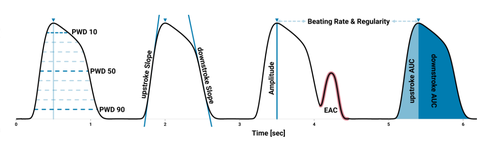
Methods

- The FLEXcyte technology is based on a special 96 well plate that contains ultra-thin and hyper-elastic silicone membranes as basis for hiPSC-CMs (Fig 1.A).
- Rhythmic contraction of cardiomyocyte (CM) monolayers lifts the membranes in the FLEXcyte 96 plate upwards. These changes in deflection are quantified by means of capacitive distance sensing with the FLEXcyte device (Nanion Technologies. (Fig.1A)).
- Parameters analyzed are contractile force (mN/mm²), rising and falling times, AUC as well as peak width duration (PWD) 10 - 90 and beat rate (Fig.1B) (Gossmann *et al.*, 2020).
- Mean beat of ventricular and atrial hiPSC-derived atrial CMs before compound addition showing chamber-specific beat shapes (Fig.1C).

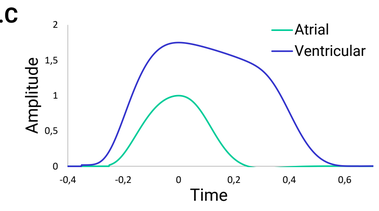
1.A



1.B



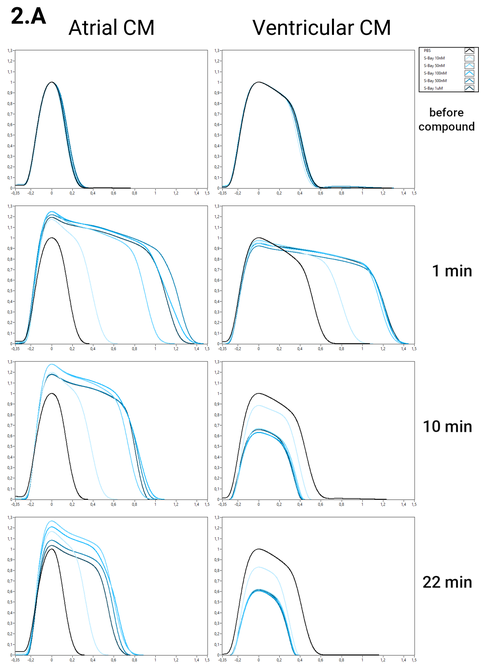
1.C



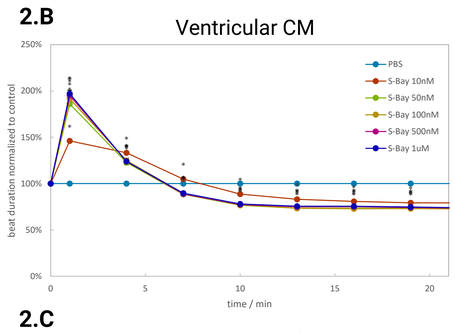
Results

S-Bay K8644

2.A



2.B



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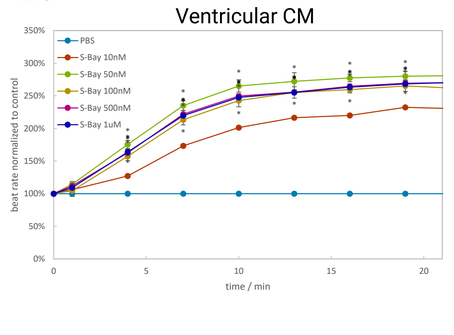
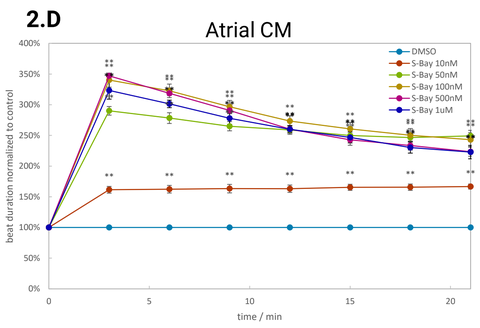


2.D



2.E

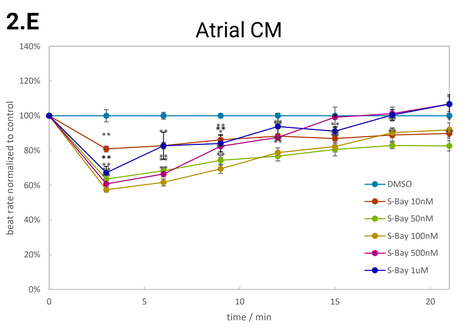
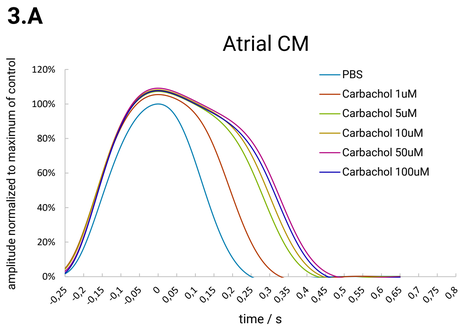


Figure 2. A Comparison of atrial and ventricular hiPSC-derived CM mean beats before compound administration as well as after 1 min, 10 min and 22 min of S-Bay K8644 treatment. Compound treatment with a concentration range of 100 nM – 1 µM was performed 5 days after seeding on FLEXcyte 96 plates. **B,C** Beat duration and beat rate of ventricular CMs after S-Bay K8644 treatment ranging from 10 nM – 1 µM. **D,E** Beat duration and beat rate of atrial CMs treated with S-Bay K8644 for 22 min. DMSO served as control condition (blue) normalized to 100% for both cell types. Asterisks represent statistical significance with $p<0.05$ (*) or $p<0.01$ (**), (Wilcoxon Mann Whitney test).

carbachol

3.A



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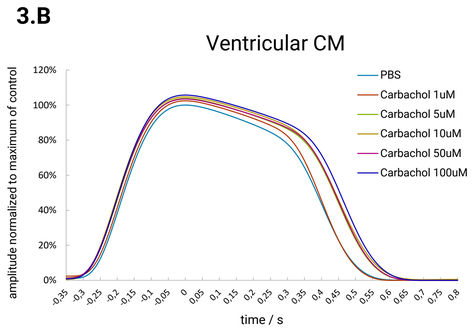

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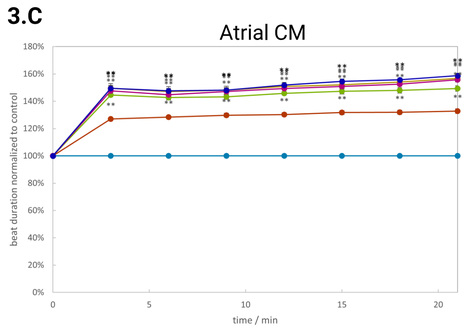

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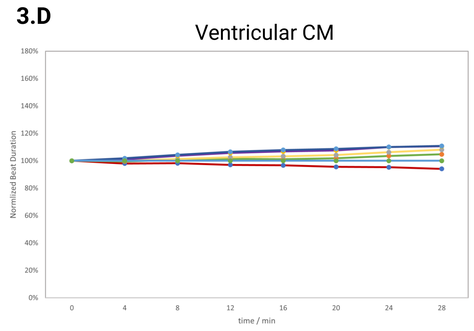

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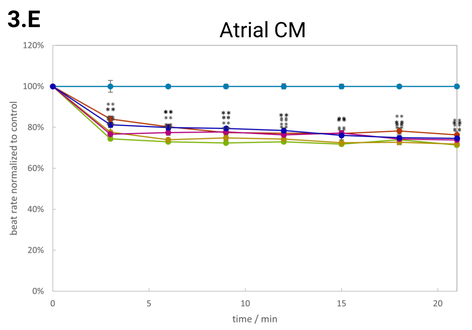


3.F

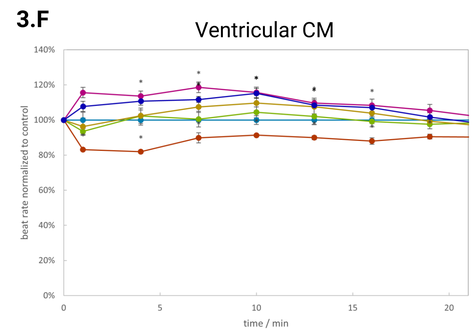
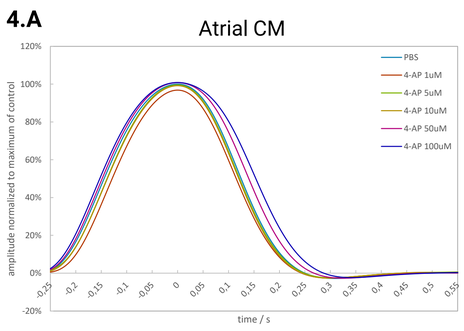


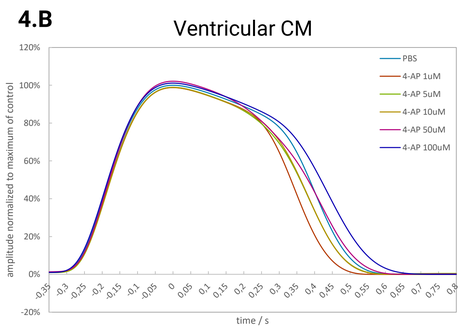
Figure 3. A,B Mean Beat of axoCells™ atrial (A) and ventricular (B) hiPSC-derived cardiomyocytes cultured on FLEXcyte 96 plates for 5 days before compound administration of carbachol 1 µM – 100 µM. Amplitudes are normalized to maximum of control. **C,E** Beat duration and beat rate of atrial CMs after treatment with carbachol normalized to control. **D,F** Beat duration and beat rate of ventricular CMs after treatment with carbachol normalized to control. Asterisks represent statistical significance with $p<0.05$ (*) or $p<0.01$ (**), (Wilcoxon Mann Whitney test).

4-AP

4.A



4.B



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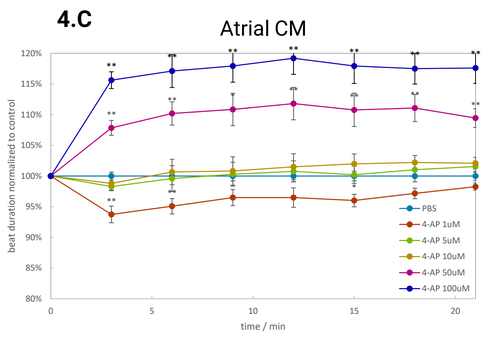

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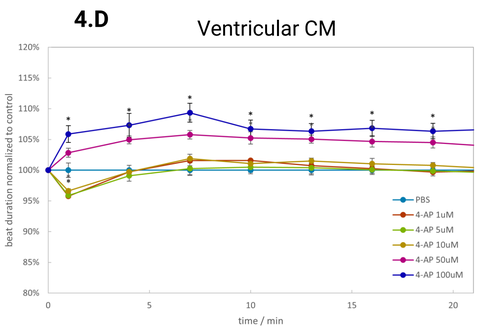

Figure 4. A,B Mean beat of atrial (A) and ventricular (B) CMs cultured on FLEXcyte 96 plates treated with 4-Aminopyridine (4-AP) ranging from 1 µM – 100 µM. **C,D** Beat duration of atrial (C) and ventricular (D) CMs treated with different 4-AP concentrations for 22 min. PBS served as control (blue) normalized to 100% for both cell types. Statistical analysis, $p<0.05$ (*) or $p<0.01$ (**), (Wilcoxon Mann Whitney test).

Summary

- Mean beats of axoCells™ hiPSC-derived ventricular and atrial cardiomyocytes displayed beat shapes analogue to the respective cardiac action potential, in which ventricular hiPSC-CMs show a calcium influx-related extended plateau phase (Grunnet et al., 2010) compared to atrial cells.
- Cells treated with L-Type calcium channel agonist S-Bay K8644 showed an immediate concentration-dependent increase in beat duration for both cell types. This effect changed for ventricular cells by a shortening in duration and an increase in beat rate over time.
- Carbachol, an agonist of atrial-specific I_{KACh}, expected to exhibit negative chronotropic effects, showed a significant increase in beat duration of atrial CMs alongside a decrease in beat rate. However, these responses were not observed to the same extent in ventricular hiPSC-CMs, demonstrating the functional activity of an atrial-specific ion channel in atrial hiPSC-CMs.
- 4-Aminopyridine (4-AP), an antagonist of the I_{Kur} ion channel, primarily found on atrial cells (Dobrev et al., 2001) showed a concentration-dependent transient effect in both cell types, but with stronger effect on atrial cells. Here, minor concentrations exhibit a decreasing effect on the beat duration while higher concentrations exhibit a duration prolongation.

Conclusions

- HiPSC-derived atrial and ventricular CMs reproduced the different contractile phenotypes and pharmacological responses of primary cardiomyocyte subtypes suitably. Hence, these cell types provide the starting point to develop more reliable, physiological-relevant research on subtype-specific cardiac diseases.
- Contractile property evaluation of ventricular and atrial CMs with the FLEXcyte technology offered the unique possibility to analyze cardiac contraction behavior under physiological conditions in a 96-well format (Gossmann *et al.*, 2020, Lickiss et al., 2022).
- The data shown here proves the combined robustness of Axol Biosciences's axoCell™ products and the FLEXcyte 96 technology for preclinical cardiac risk assessment.

Dobrev D. et al., Circulation. 2001
Grunnet M. et al. Acta Physiologica. 2010
Gossmann M. et al. J. of Pharm. and Tox. Methods. 2020
Lickiss. et al. Journal of Visualized Experiments. 2022