PC006 Functional and pharmacological differences between the contractility of axoCells™ iPSC-derived atrial and ventricular cardiomyocytes assessed on the FLEXcyte 96

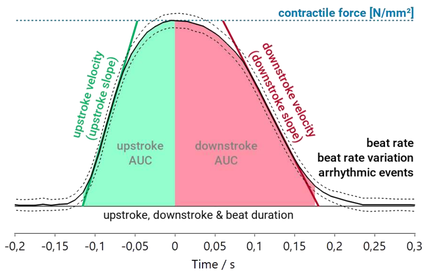
Steven D. Broadbent[1] (steven.broadbent@axolbio.com), Bettina Lickiss[2], Matthias Gossman[2], Peter Linder[2], Jamie R. Bhagwan[1], Ashley Barnes[1].

1. Axol Bioscience Ltd., Babraham Research Campus, B260 Meditrina Building, Cambridge, CB22 3AT, UK
2. innoVitro GmbH, Artilleriestr. 2, 52428 Jülich, Germany

Overview and Introduction

Commercial human iPSC-derived ventricular cardiomyocytes have been available for over a decade and are used in research, drug development, and toxicology testing. Less work has been performed on human iPSC-derived atrial cardiomyocytes despite the clear phenotypic and pharmacological differences between atrial and ventricular cardiomyocytes.

Atrial fibrillation (AF) is the most common form of arrhythmia worldwide, affecting over 33M people, and is a co-morbidity with obesity, stroke, dementia, and congestive heart failure. There are currently limited therapeutic options with poor success rates and a 1-year mortality rate of 25%. With a clear need for better, more human models of AF, Axol have partnered with innoVitro GmBH to examine the phenotypic and pharmacological differences in contractility of axoCells atrial and ventricular cardiomyocytes on their FLEXcyte 96 platform.



Methods

Axol's axoCells atrial (ax2518) and ventricular (ax2508) human iPSC-derived cardiomyocytes were cultured according to Axol's user guides. The cells were cultured on fibronectin-coated FLEXcyte 96 well plates in 200 µL cardiac maintenance medium (ax2530) per well. Cells were seeded approximately 6 days before compound treatment at 65k per well (195,000 cells/cm^2) to allow proper monolayer and network formation. A final media change was conducted 4-6 hours before drug application. For the experiments, 50 µL for the cell culture medium was removed and replaced with 50 µL medium containing 4x concentrated compound, resulting in the desired final compound concentration. All compounds and concentrations were tested at n=4 and assessed using the Wilcoxon rank-sum test. The FLEXcyte 96 enables the analysis of several contractility parameters including contractile force, beat rate, and beat duration.

Waveform (Baseline)

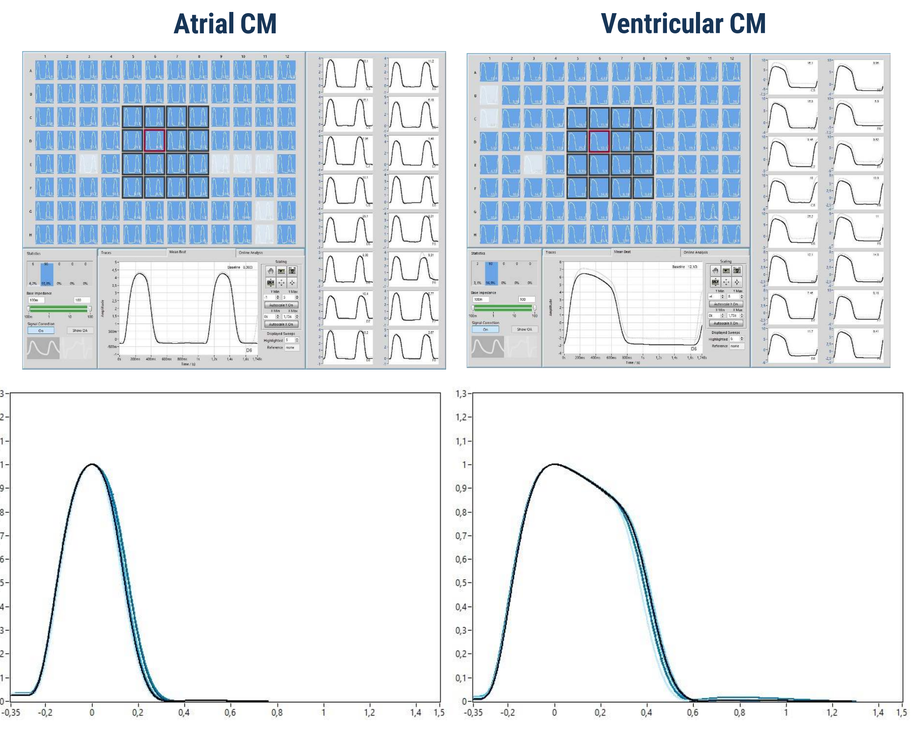


Figure 1. The Flexcyte 96 platform shows that axoCells hiPSC-derived atrial and ventricular cardiomyocytes demonstrate distinct beat rates and contractility waveforms consistent with chamber-specific primary cardiomyocytes.
The Flexcyte 96 allows the measurement of the contractility of hiPSC-derived cardiomyocytes. By day 6, across the Flexcyte 96 plates (**Top**) consistent and distinct waveforms were seen in each well depending on whether the plates were seeded with axoCells hiPSC-derived atrial or ventricular cardiomyocytes, with atrial cells having a higher beat rate. Zooming into a single representative well the atrials (**Bottom Left**) can be seen to produce a shorter, more rounded waveform while the ventriculars (**Bottom Right**) produce a longer-lasting waveform with a more pronounced plateau. This is consistent with data reported with primary cardiomyocytes (Grunnet, 2010) and is thought to be due to ventricular cardiomyocytes experiencing a greater Ca^{2+} influx through L-type Ca^{2+} channels than atrials, prolonging the contraction.

Pharmacology (Beat Rate)

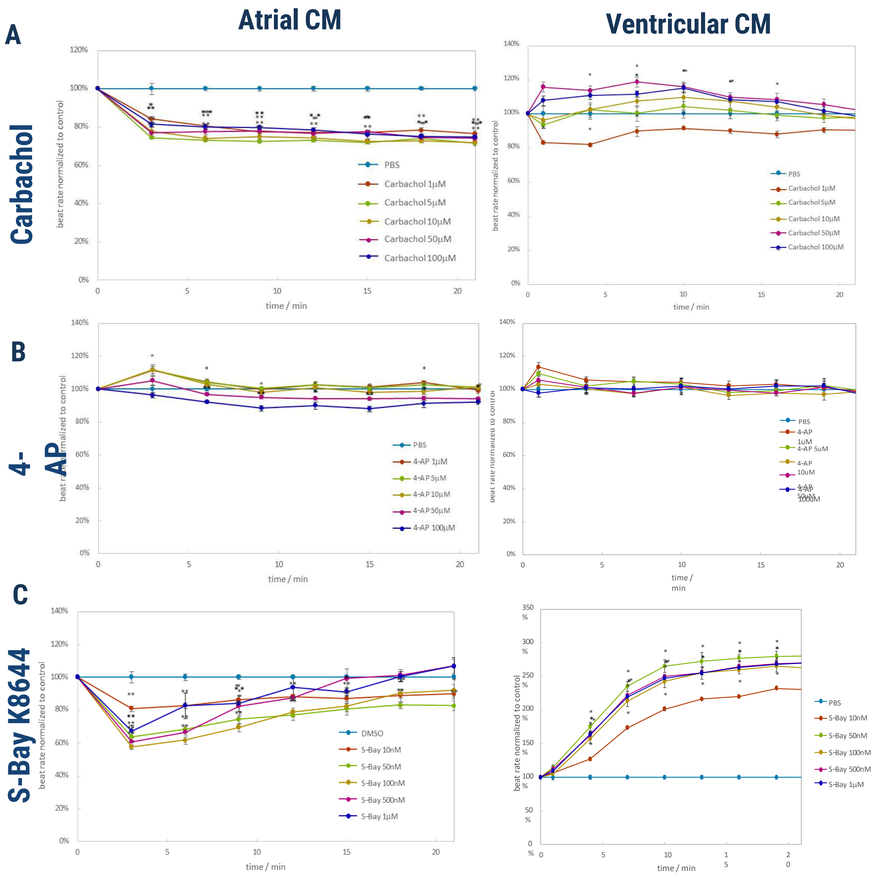


Figure 2. axoCells hiPSC-derived atrial and ventricular cardiomyocytes correctly reproduce the chamber-specific pharmacology of primary cardiac cells.
Carbachol, (**A**) which activates the atrial-specific acetylcholine-activated inward-rectifying potassium current produced a significant decrease in beat rate in atrial cardiomyocytes while actually increasing beat rate in ventricular cardiomyocytes at the highest concentrations. Whereas the highest concentrations of 4-AP (**B**), inhibitor of the atrial-specific ultra-rapid delayed rectifier potassium channels, decreased the beat rate of atrials while having no effect on ventricular cardiomyocytes. In comparison the L-type calcium channel agonist S-Bay K8644 (**C**) transiently decreased beat rate in atrials while producing a marked and sustained increase in beat rate in ventricular cardiomyocytes.

Pharmacology (Beat Duration)

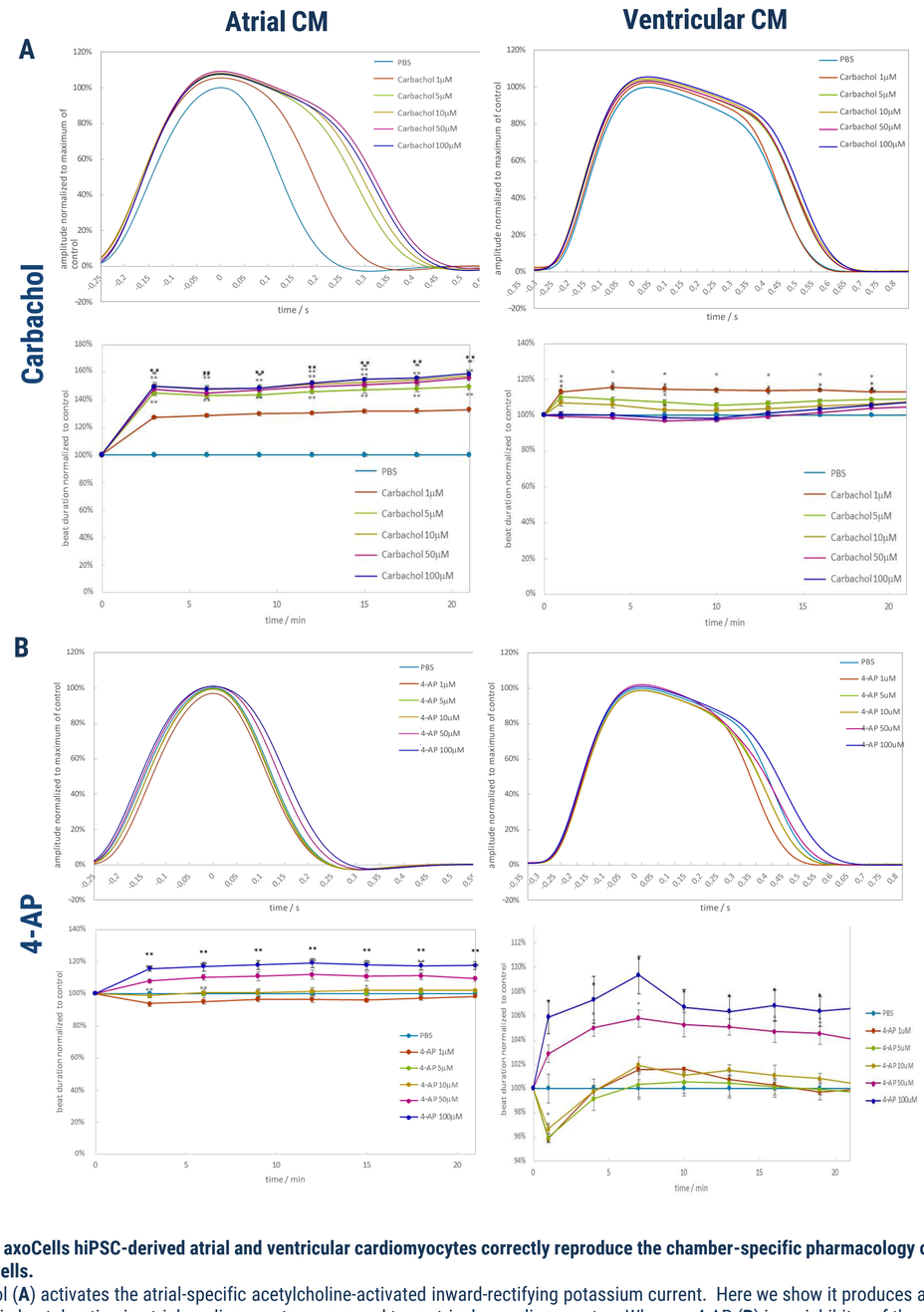


Figure 3. axoCells hiPSC-derived atrial and ventricular cardiomyocytes correctly reproduce the chamber-specific pharmacology of primary cardiac cells.
Carbachol (**A**) activates the atrial-specific acetylcholine-activated inward-rectifying potassium current. Here we show it produces a far greater increase in beat duration in atrial cardiomyocytes compared to ventricular cardiomyocytes. Whereas 4-AP (**B**) is an inhibitor of the atrial-specific ultra-rapid delayed rectifier potassium channels produced an increased beat duration in atrial cardiomyocytes but an actual transient decrease in ventricular cardiomyocytes. Both these results demonstrate that atrial-specific ion channels are present in greater levels axoCells hiPSC-derived atrial cardiomyocytes compared to axoCells ventricular cardiomyocytes.

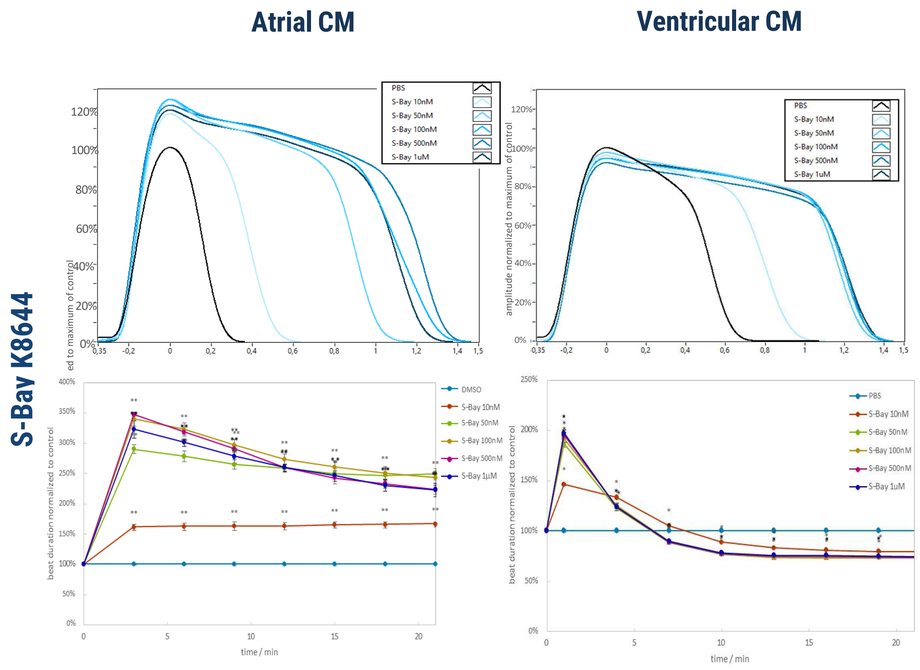


Figure 4. axoCells hiPSC-derived atrial and ventricular cardiomyocytes reveals chamber-specific pharmacology of S-Bay K8644.
The L-type calcium channel agonist S-Bay K8644 increased plateau phase (**Top**) in both atrial and ventricular cardiomyocytes increasing beat duration in a dose-dependent manner. This is as predicted and is due to calcium-induced calcium release from the sarcoplasmic reticulum Ca^{2+} stores. The increased beat duration caused by S-Bay K8644 tends to be transient and rapidly declines after drug addition in both atrial and ventricular cardiomyocytes (**Bottom**). There are marked chamber-specific differences though, with the atrial increase being sustained for a prolonged period (albeit at a lower level) whereas the ventricular cells rapidly return to baseline levels within 10 minutes before undershooting. This demonstrates the discriminatory power of using axoCells chamber-specific cardiomyocytes as a platform to investigate the pharmacology and toxicology of novel compounds.

Conclusion

The innoVitro Flexcyte 96 contractility platform has demonstrated how axoCells human iPSC-derived atrial (ax2518) and ventricular cardiomyocytes (ax2508) can be used to correctly reproduce the different phenotypes and pharmacology of chamber-specific primary cardiomyocyte sub-types.

This supports previous work by Axol and collaborators showing chamber-specific phenotypes, electrophysiology, and pharmacology through ICC, RNAseq, manual patch clamp, and Multi-Electrode Array using our axoCells human iPSC-derived atrial and ventricular cardiomyocytes.

Therefore, the use of chamber-specific hiPSCs on platforms such as the Flexcyte 96 provides the starting point to develop more reliable, human physiologically-relevant models for research on atrial cardiomyocytes and AF as well as broader research on cardiac electrophysiology, pharmacology, and toxicology.

References and Further Information

- 1. Grunnet, M. 2010. Repolarization of the cardiac action pot *Acta Physiol* 198 Suppl 676:1-48

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