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

Commercial human iPSC-derived cardiomyocytes (hiPSC-CMs) have been available from multiple providers for over a decade, with extensive use in research, drug development and toxicology testing due to their appropriate modelling of primary human ventricular cardiomyocytes. Less work has been performed on human iPSC-derived atrial cardiomyocytes despite the clear phenotypic and pharmacological differences between atrial and ventricular cardiomyocytes, rendering the standard ventricular cardiomyocytes a poor model for atrial research. This is of particular concern as atrial fibrillation (AF) is the most common form of arrhythmia worldwide affecting over 33M people and rising, being a co-morbidity with obesity, stroke and congestive heart failure (Chugh *et al.*, 2014). Despite the clear clinical need for better AF treatments there are limited therapeutic options with poor success rates and the 1-year mortality rate for patients with AF remains around 25% (Lee *et al.*, 2018). Therefore, there is a clear need for better, more human models of AF.

Axol Bioscience Ltd have developed their axoCells™ iPSC-derived atrial cardiomyocytes (ax2518) and have performed extensive validation studies showing differences in key markers, such as MLC2A, ANP and KCNA5 and electrophysiology between their isogenic atrial and ventricular cardiomyocytes (ax2508). There has been less investigation carried out on the contractility differences between the two cell types which is of particular relevance to AF. Therefore, Axol have partnered with innoVitro GmbH to examine the phenotypic and pharmacological differences in contractility of Axol's two forms of cardiomyocytes on their FLEXcyte 96 platform.

With less than 10 µm in thickness and sophisticated surface modification, the polydimethylsiloxane (PDMS) membranes of the FLEXcyte 96 disposable plates offer physiological elasticity of native human heart tissue and strong mechanical support. While being deflected by the weight of the culture medium, rhythmic contraction of the cardiomyocytes lifts the membranes in the 96-well upwards. By measuring the changes in deflection, mechanical stress can be calculated.

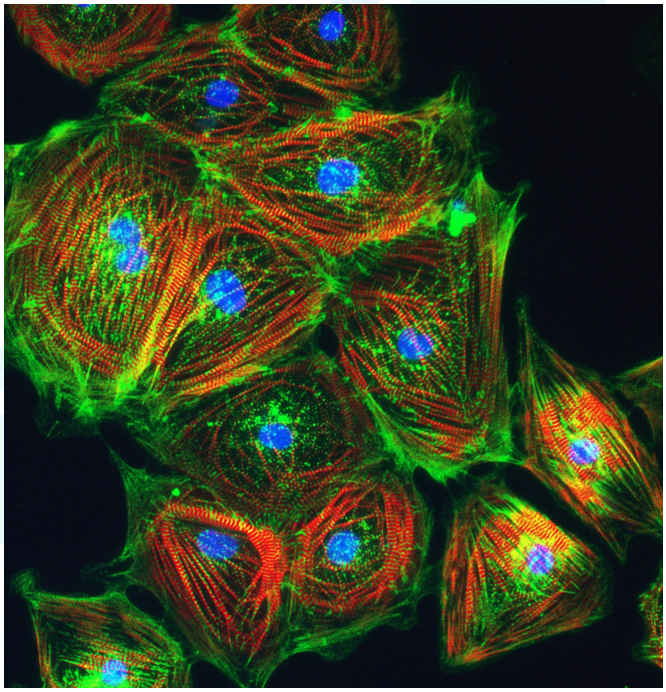
The L-type calcium channel agonist S-Bay K8644 is known for its positive inotropic effects on the human heart, and also for prolonging the plateau phase of the cardiac action potential.

The atrial-specific drugs Carbachol and 4-Aminopyridine (4-AP) act on atrial-specific ion channels. Carbachol, an agonist of I_{KACh}, is expected to exhibit negative chronotropic effects. 4-AP, an antagonist of I_{Kur}, is expected to shorten the action potential duration.

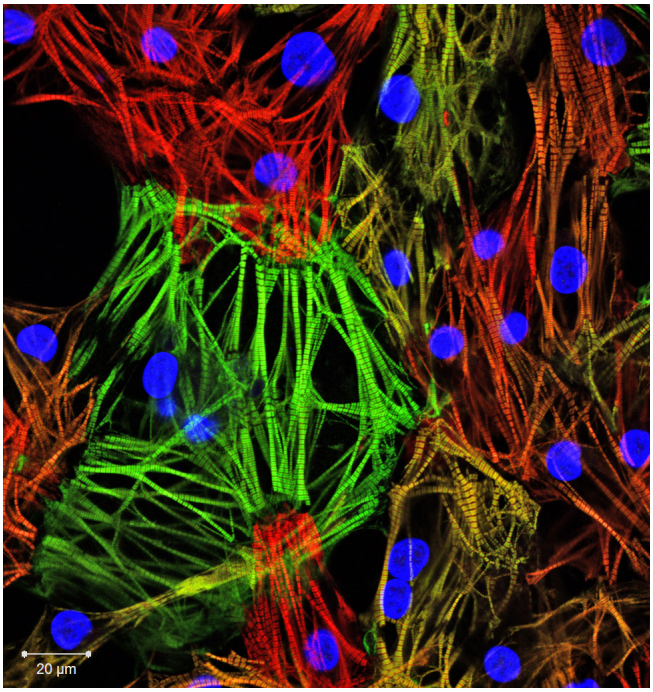
Here, we show that Carbachol, 4-AP and S-Bay K8644 produced distinctly different effects on axoCells™ ventricular [ax2508] and atrial [ax2518] hiPSC-CMs in terms of waveform shape, beat rate and beat duration.

Therefore, axoCells™ human iPSC-derived atrial and ventricular cardiomyocytes correctly reproduced the different phenotypes and pharmacology of primary cardiomyocyte sub-types as assessed using the FLEXcyte 96 and, as such, provide the starting point to develop more reliable, physiological-relevant models for research on atrial cardiomyocytes and AF.

AXOL



Cardiac Troponin T / Cardiac alpha Actinin / DAPI



MLC2V / MLC2A / DAPI

Figure 1. Immunocytochemistry characterisation of axoCells™ human iPSC-derived ventricular cardiomyocytes [ax2508]. Cardiac troponin T and cardiac alpha actinin are pan-cardiac, sarcomeric proteins. MLC2V is the ventricular isoform of the regulatory light chain of myosin, whilst MLC2A is the atrial isoform.

Methods

Both atrial [ax2518] and ventricular [ax2508] human iPSC-derived cardiomyocytes were provided by Axol Bioscience Ltd (Edinburgh, UK).

The cells were cultured on fibronectin-coated FLEXcyte 96 well plates according to manufacturers' guidelines in 200 µL maintenance medium 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.

The FLEXcyte 96 enables the analysis of a number of contractility parameters including contractile force, beat rate and beat duration (Figure 2).

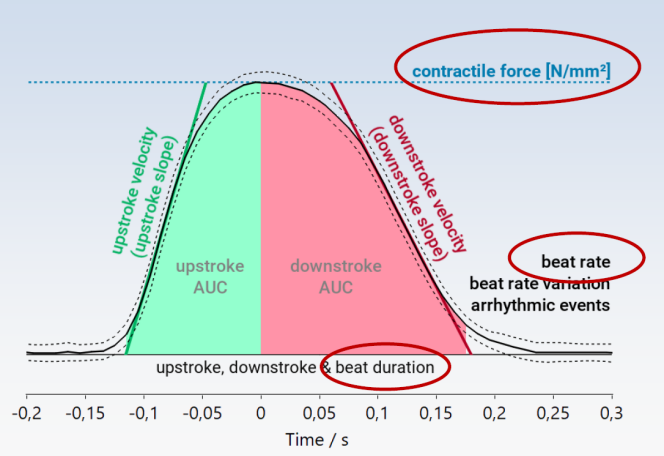


Figure 2. FLEXcyte 96 contractility parameters

Results

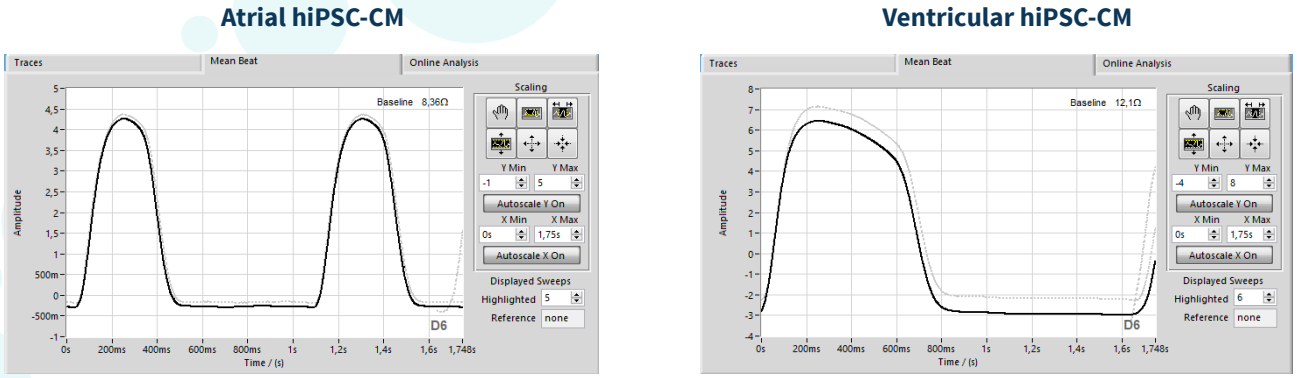


Figure 3. Representative waveforms of axoCells™ atrial and ventricular hiPSC-CMs on the FLEXcyte 96 platform recorded after 6 days

Six days post-thaw, atrial and ventricular hiPSC-derived cardiomyocytes exhibited distinct contractility waveforms on the FLEXcyte 96 platform (Figure 3). Atrial hiPSC-CMs showed a more rounded waveform, whereas ventricular hiPSC-CMs showed a slightly more prolonged plateau phase. This is similar to the cardiac action potential, in which ventricular cardiomyocytes have an extended plateau phase compared to atrial cardiomyocytes due to greater Ca^{2+} influx through L-type Ca^{2+} channels (Grunnet, 2010).

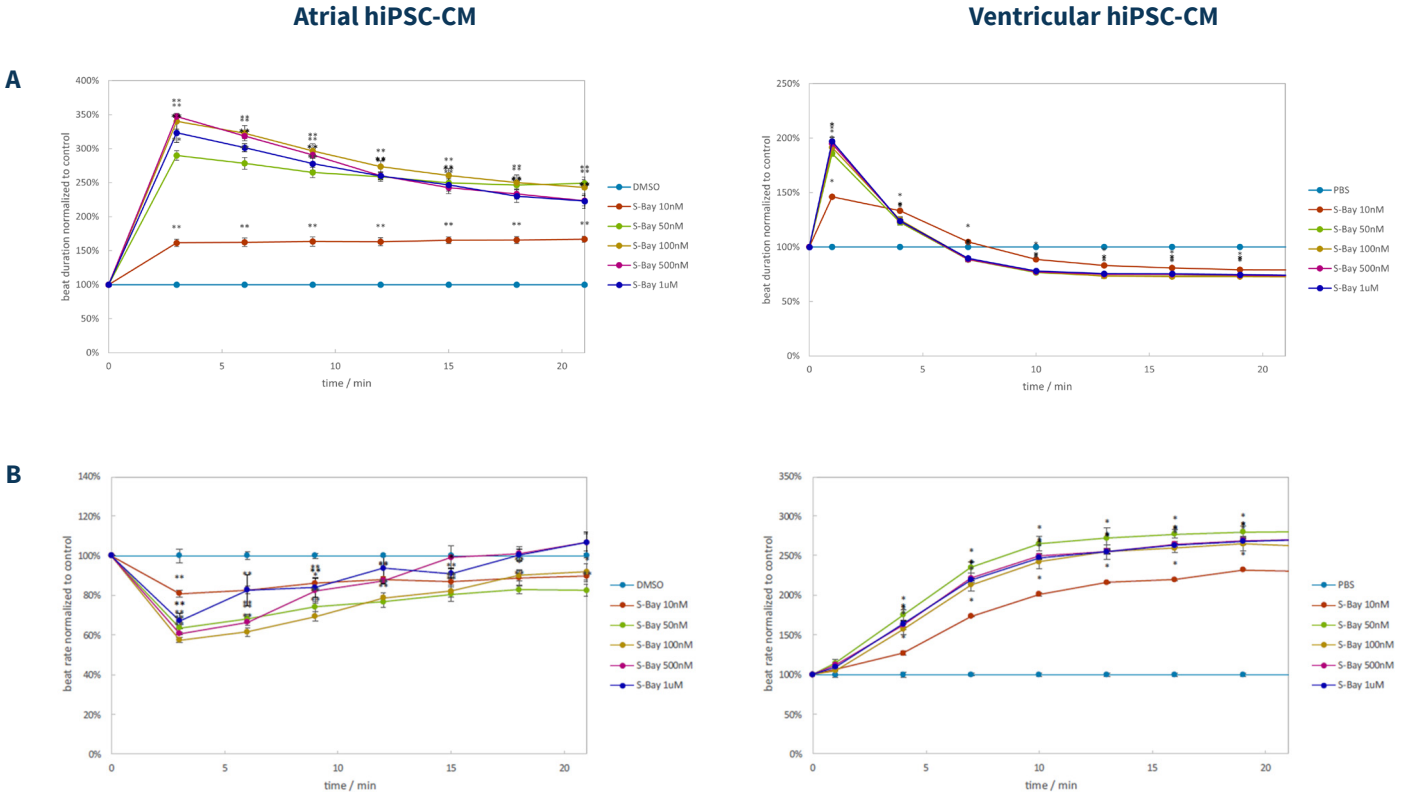


Figure 4. The effect of S-Bay K8644, an L-type Ca^{2+} channel agonist, on contractility of axoCells™ atrial and ventricular hiPSC-CMs. (A) Beat duration. (B) Beat rate.

S-Bay K8644 is an L-type Ca^{2+} channel agonist and can exhibit a positive inotropic effect and prolonged plateau phase 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 is seen in the first 5 minutes after drug addition in both atrial and ventricular hiPSC-CMs (Figure 4A). However, beat rate transiently decreases in atrial hiPSC-CMs, whereas a sustained increase in beat rate is observed in ventricular hiPSC-CMs (Figure 4B). These transient effects may be due to the calcium stores in the sarcoplasmic reticulum becoming rapidly depleted.

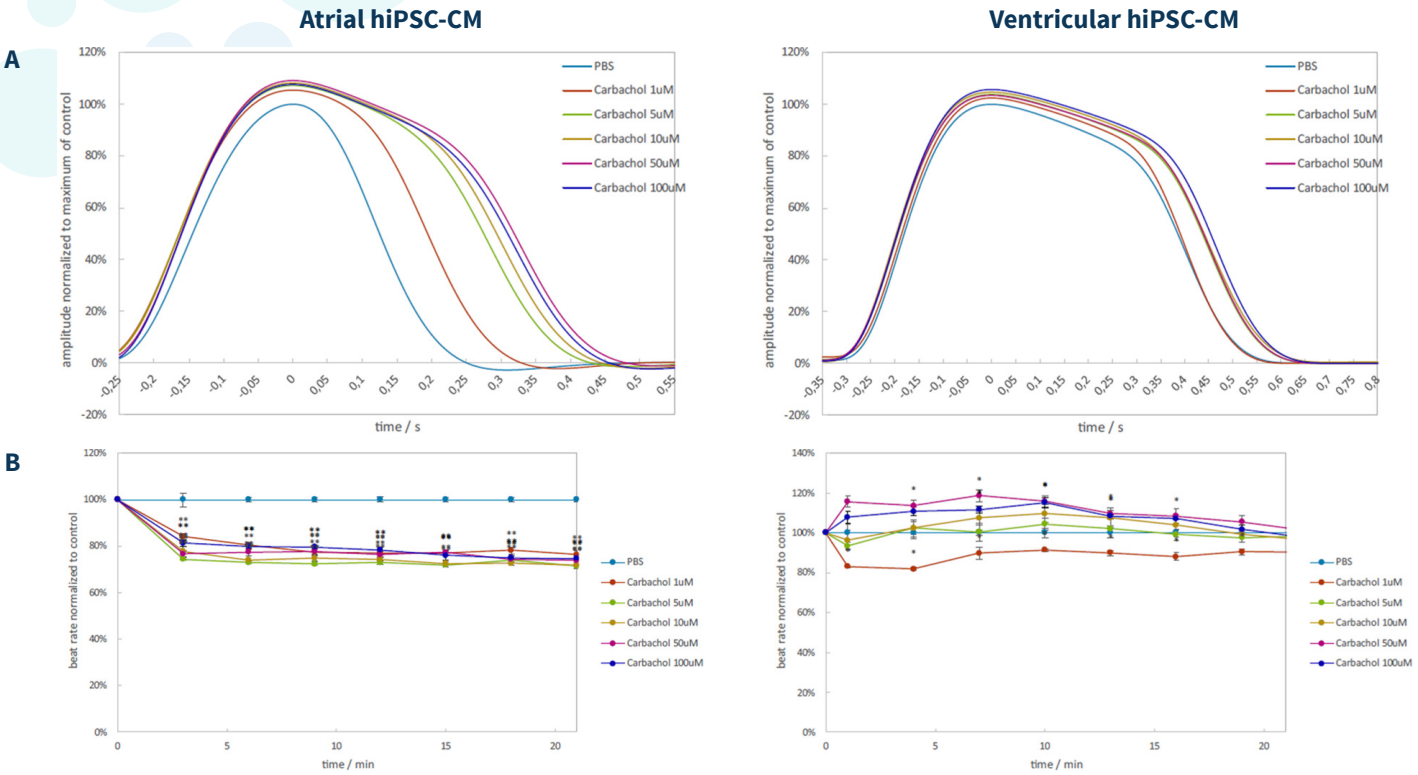
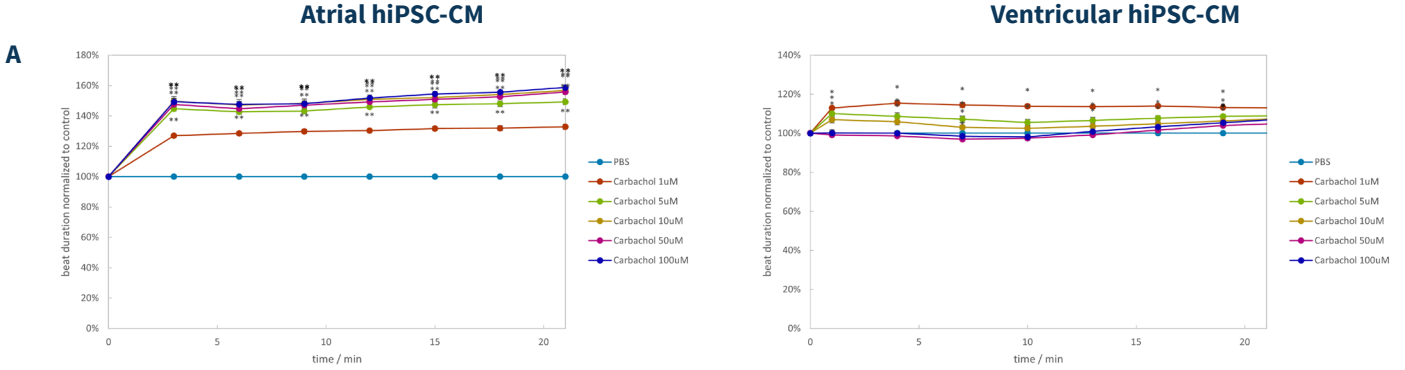


Figure 5. The effect of Carbachol, an activator of I_{KACh} in atrial cardiomyocytes, on the contractility of axoCells™ atrial and ventricular hiPSC-CMs. (A) Mean beat. (B) Beat rate



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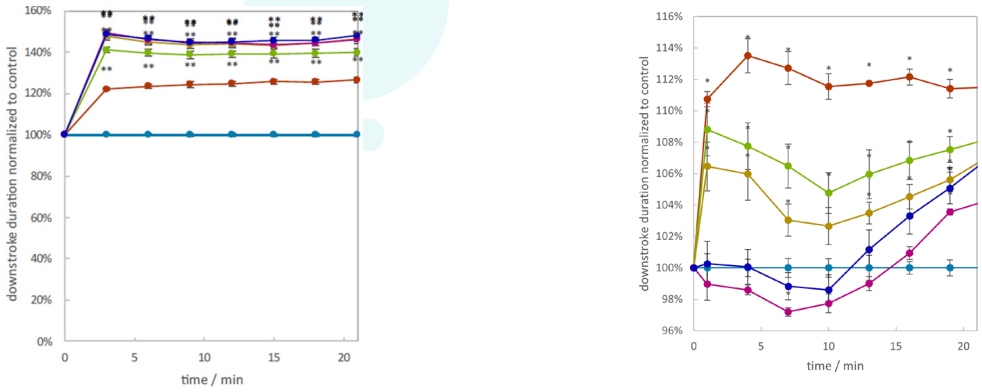


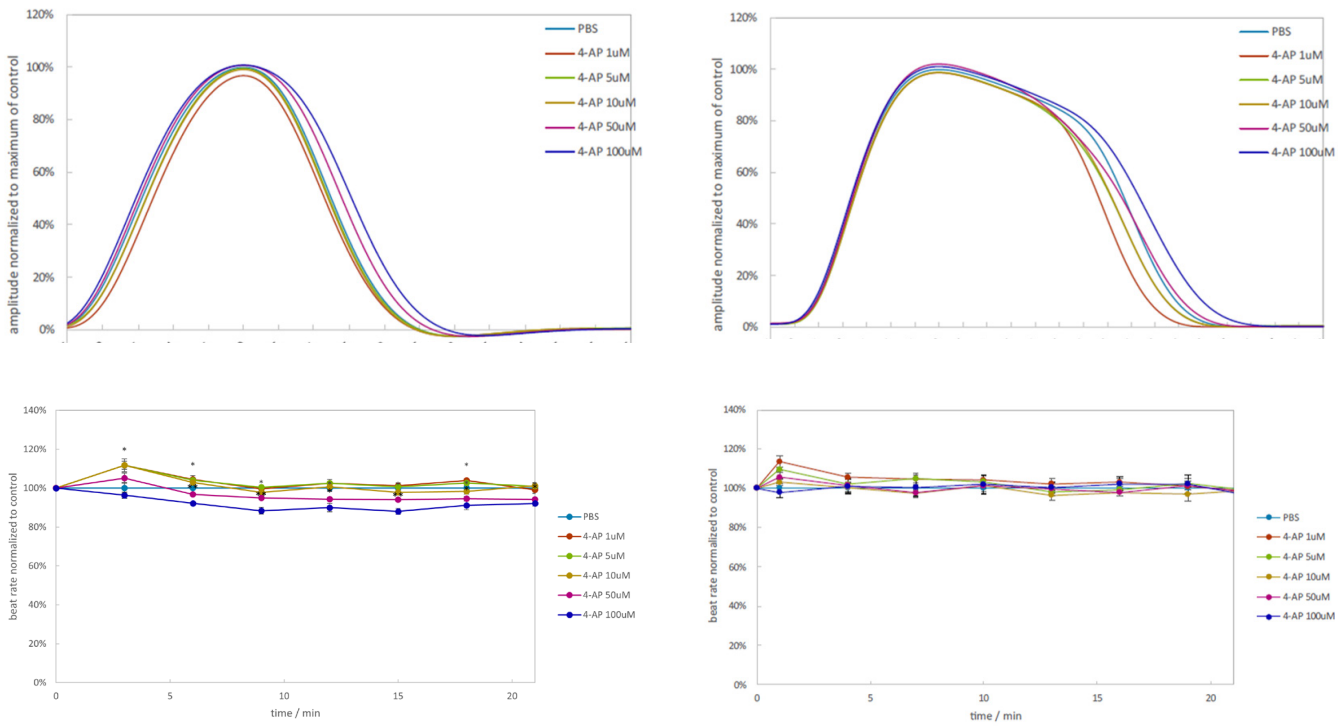
Figure 6. The effect of Carbachol, an activator of I_{KACh} in atrial cardiomyocytes, on the contractility of axoCells™ atrial and ventricular hiPSC-CMs. (A) Beat duration. (B) Downstroke duration

The acetylcholine-activated inward-rectifying potassium current (I_{KACh}) is primarily found on atrial cardiomyocytes (Heijman *et al.*, 2018), and its activation by Carbachol is observed to a greater extent in axoCells™ atrial hiPSC-CMs compared to axoCells™ ventricular hiPSC-CMs (Figure 5, 6). At all concentrations tested, carbachol exhibited a negative chronotropic response and prolonged beat duration on axoCells™ atrial hiPSC-CMs. However, these responses were not observed to the same extent in axoCells™ ventricular hiPSC-CMs, demonstrating the functional activity of an atrial-specific ion channel in atrial hiPSC-CMs.

Atrial hiPSC-CM **Ventricular hiPSC-CM**

A

B



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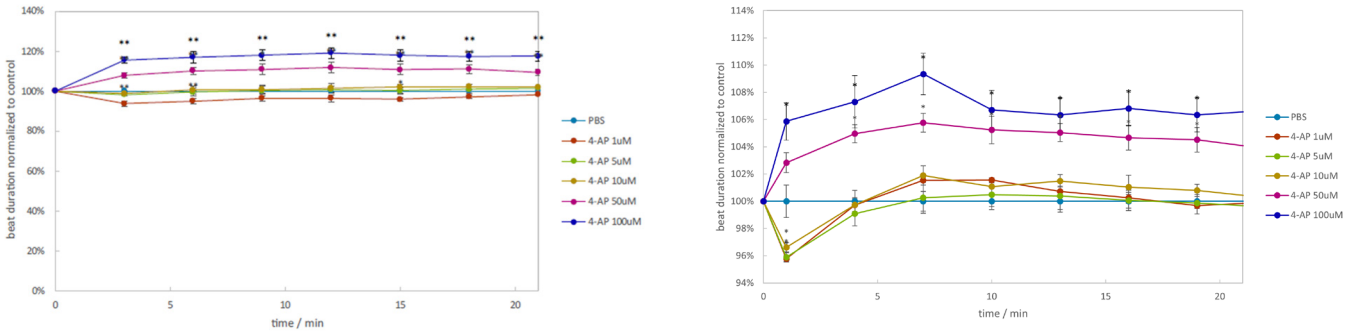

Figure 7. The effect of 4-AP, an inhibitor of I_{Kur} in atrial cardiomyocytes, on the contractility of axoCells™ atrial and ventricular hiPSC-CMs. (A) Mean beat. (B) Beat rate. (C) Beat duration.

4-Aminopyridine (4-AP) inhibits the ultra-rapid delayed rectifier potassium channel (I_{Kur}), primarily found on atrial cardiomyocytes (Dobrev *et al*., 2001). When applied to axoCells™ atrial hiPSC-CMs, 4-AP caused a reduction in beat rate and an increased beat duration, albeit at higher concentrations of 50 µM and 100 µM (Figure 7). In contrast, axoCells™ ventricular cardiomyocytes showed no change in beat rate and a transient reduction in beat duration at 1 µM, 5 µM and 10 µM. Again, this demonstrates the presence of functional atrial-specific ion channels on axoCells™ atrial hiPSC-CMs, that are not present to the same extent on axoCells™ ventricular hiPSC-CMs.

Conclusion

Here we show that distinct pharmacological effects can be observed between axoCells™ atrial and ventricular hiPSC-derived cardiomyocytes when using the FLEXcyte 96 platform to assess contractility.

Activation of I_{KACh} by Carbachol reduced beat rate and prolonged beat duration in atrial cardiomyocytes but not ventricular cardiomyocytes. In addition, inhibition of I_{Kur} by 4-AP reduced beat rate and prolonged beat duration in atrial cardiomyocytes but not ventricular cardiomyocytes, demonstrating the inhibition of Phase I repolarisation in atrial cardiomyocytes.

Therefore, the presence of functional I_{KACh} and I_{Kur} currents in axoCells™ atrial cardiomyocytes that respond appropriately to drugs provides a readily available commercial resource for the *in vitro* modelling of atrial-specific disorders such as atrial fibrillation. In combination with the simultaneous multiwell measurements of a range of contractility parameters on the FLEXcyte 96 platform, this provides a powerful tool to perform *in vitro* drug screening and disease modelling on subtype-specific hiPSC-derived cardiomyocytes.

References

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