New Approach Methodologies (NAMs): assessing cardiotoxicity and neurotoxicity *in vitro* using human iPSC-derived cells

Jamie R. Bhagwan[1] (jamie.bhagwan@axolbio.com), David Wallbank[1], Jan Hunker[2], Bettina Lickiss[2], Steven D. Broadbent[1], Sonja Stoelze-Feix[3] , Matthias Gossmann[2], Sapna Vyas[1]
1. Axol Bioscience Ltd., Babraham Research Campus, B260 Meditrina Building, Cambridge, CB22 3AT, UK. 2. innoVitro GmbH, Artilleriestr. 2, 52428 Jülich, Germany. 3. Nanion GmbH, Ganghoferstr 70A, 80339 Munich, Germany. 4. Imperial College London, ICTEM Building, Hammersmith, W12 0NN, UK

Abstract

New approach methodologies (NAMs) are gaining traction for drug toxicity screening. In recent years, human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) and hiPSC-derived sensory neurons have found greater utility for *in vitro* toxicity screens. They are a scalable, physiologically relevant model system, providing an ethical alternative to animal testing. Here, we show the application of commercially available hiPSC-derived cells on multiwell plate formats to assess drug response and toxicity.

Contractility assessment using the FLEXcyte™ 96 system showed that chamber-specific hiPSC-derived cardiomyocytes exhibited different baseline contractility waveforms. Atrial cardiomyocytes had a faster beat rate, shorter beat-width and lower contractile amplitude when compared to ventricular cardiomyocytes. In addition, atrial and ventricular cardiomyocytes demonstrated varying responses to the compounds tested. Of note, the Ca^{2+} agonist S-Bay K8644 had opposing effects on chamber-specific human iPSC-CMs, with atrial cardiomyocytes exhibiting transiently reduced beat rate, increased contractile force and longer beat duration whereas ventricular cardiomyocytes exhibited increased beat rate, decreased contractile force and shorter beat duration.

Neurite outgrowth assessment of hiPSC-derived sensory neurons using the Incucyte® S3 demonstrated that effective cell masking could be achieved using the NeuroTrack module, albeit with higher seeding densities reducing the accuracy of neurite tracking. Paclitaxel treatment inhibited neurite outgrowth, confirming assay sensitivity to neurotoxic compounds. Application of paclitaxel at day 7 produced a larger assay window at neuronal maturity (day 21) compared with treatment initiated at day 14.

Taken together, these results illustrate the utility of NAMs as a physiologically relevant, scalable *in vitro* model for assessing drug response and toxicity.

Results

Characterization of axoCells™ human iPSC-derived cardiomyocytes

Both axoCells™ atrial and axoCells™ ventricular cardiomyocytes are manufactured from the same 74-year-old, male donor, under ISO9001:2015 quality standards. QC by immunocytochemistry, performed 7 days post-thaw, shows a higher MLC2V positive population in axoCells™ ventricular cardiomyocytes (Figure 1A). A greater MLC2A positive population is observed in axoCells™ atrial cardiomyocytes (Figure 1B).

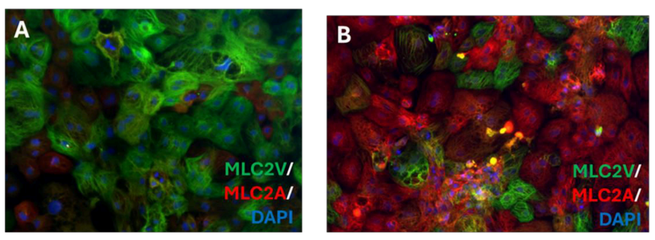
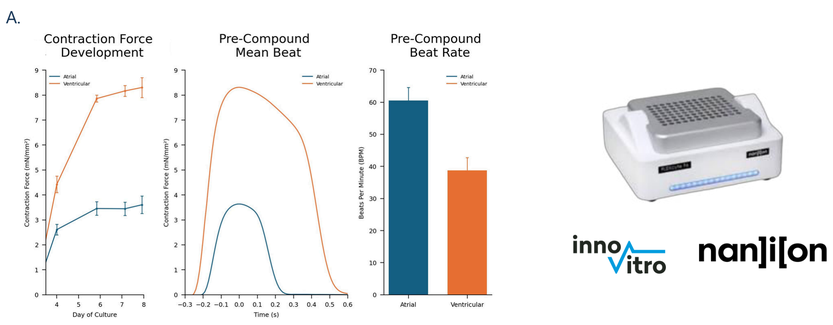


Figure 1. (A) axoCells™ ventricular cardiomyocytes and (B) axoCells™ atrial cardiomyocytes immunostained for MLC2V (green), MLC2A (red), DAPI (blue) 10X magnification.

Chamber-specific contractility response of cardiomyocytes using FLEXcyte™ 96

The FLEXcyte96™ system (Nanion Technologies) offers the simultaneous measurement of contractility in a multi-well format. Spontaneous beating of axoCells™ ventricular or atrial cardiomyocytes causes the movement of a PDMS membrane at physiological kPa of 30, which in turn can give a direct contractility measurement (mN/mm^2). Baseline measurements showed that atrial cardiomyocytes beat with lower force amplitude, shorter beat-width and faster beat rate. A range of drugs were applied to both ventricular and atrial cardiomyocytes, with each drug tested at five different concentrations (InnoVitro). The cholinergic agonist, carbachol, elicited an atrial-specific response due to the I_{KACh} current being found predominantly in the atria. An opposing transient response to the Ca^{2+} channel agonist S-Bay K8644 was observed. This highlights that chamber-specificity can impact pharmacological response.

Chamber-specific contractility waveform differences



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Atrial-specific response to Carbachol (activator of I_{KACh})

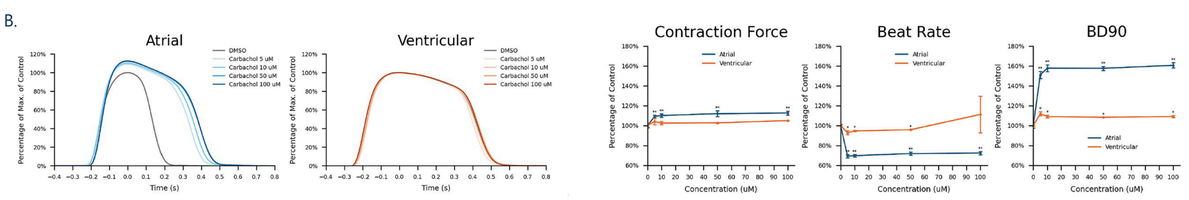

Figure 2. (A) axoCells™ ventricular (orange) and atrial (blue) cardiomyocytes baseline contractility (mN/mm^2) waveform on the FLEXcyte96™. (B) Atrial-specific response to the cholinergic agonist carbachol shows reduced beat rate and increased beat duration 90 (BD90).

Cardiotoxicity assessment of several compounds on atrial or ventricular hiPSC-derived cardiomyocytes

Compound	Contraction Force mN/mm²		Beat Rate bpm		Beat Duration sec		Upstroke Duration sec		Downstroke Duration sec		Upstroke Slope mN/mm² / sec		Downstroke Slope mN/mm² / sec		Upstroke AUC mN/mm² · sec		Downstroke AUC mN/mm² · sec		Arrhythmic Events	
	A	V	A	V	A	V	A	V	A	V	A	V	A	V	A	V	A	V	A	V
pre compound	3,63	8,31	60,50	38,70	0,40	0,74	0,18	0,23	0,22	0,51	34,50	68,94	-29,61	-41,05	0,32	1,20	0,67	2,87		
(SD)	0,82	1,29	4,20	3,66	0,03	0,05	0,02	0,01	0,02	0,05	7,67	10,52	7,21	6,61	0,08	0,21	0,19	0,64		
Acetylcholine (100 µM)	103%	98%	90% **	93%	117% **	105%	106% **	88%	123% **	109 % *	100%	105%	96%	108%	112% **	70%	130% **	121% *	×	×
Carbachol (100 µM)	113% **	105%	72% **	111% *	161% **	109% *	91% **	106% *	230% **	110% *	122% **	98%	84% **	96%	103%	111% *	282% **	117% *	✓	×
S-Bay K8644 (1 µM)	120% **	75% *	105%	230% *	217% **	91%	64%	106%	381%	73%	169%	91%	92%	82%	77%	83%	554%	54%	×	×
Ivabradine (10 µM)	75% **	76% **	67% **	85% **	122% **	112% **	92% **	86% *	138% **	130% **	78% **	67% **	39% **	26% **	67% **	60% **	83% *	75% *	×	×
Vernakalant (30 µM)	61% **	66% **	89% **	117% **	114% **	95% **	93% *	88% **	124% **	98 % *	59% **	61% **	35% **	27% **	55% **	53% **	57% **	48% **	×	×
4-AP (100 µM)	103% *	101%	88% **	97%	120% **	108% *	103%	95%	132%	113% *	99%	100%	92% *	94% *	105%	93%	138%	113% *	×	×

Table 1. Heatmap of atrial (A) and ventricular (V) hiPSC-CM responses before and after compound addition. Data shown for 20 minutes post-compound addition in all cases. Data shown as percentage change from pre-compound. n > 4 wells per compound. $p < 0.05$ (*) or $p < 0.01$ (**) (Wilcoxon-Mann-Whitney test).

Characterization of axoCells™ human iPSC-derived sensory neurons

axoCells™ sensory neuron progenitors were matured over 21 days to mature sensory neurons. Functional response was assessed using multielectrode array (MEA). At full maturity, neurons exhibited burst events in response 1 µM or 10 µM capsaicin.

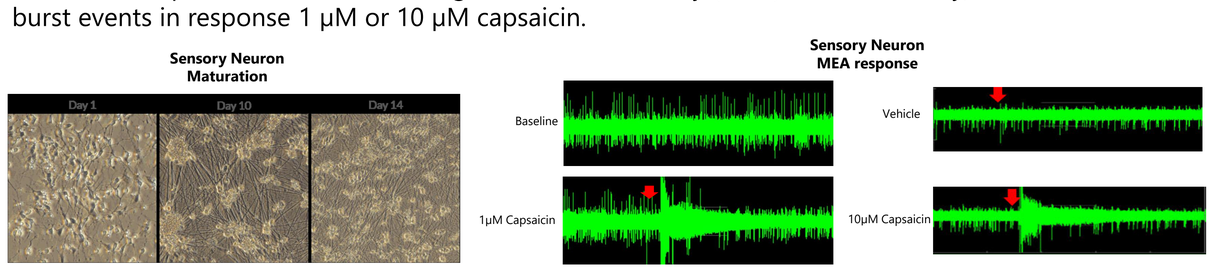

Figure 3. Brightfield images of sensory neuron maturation illustrating the growth of neurites over 21 days. Functional characterization of sensory neurons using MEA, showing burst event activity in response to capsaicin.

Neurite outgrowth assay using Incucyte® S3 live imaging

Cell masking optimization

Neurite outgrowth was quantified using the Neurotrack module and live-cell imaging over consecutive days on the Incucyte® S3 platform (Sartorius). Image analysis parameters were optimized to enable accurate segmentation of neuronal cell bodies (yellow) and neurites (pink).

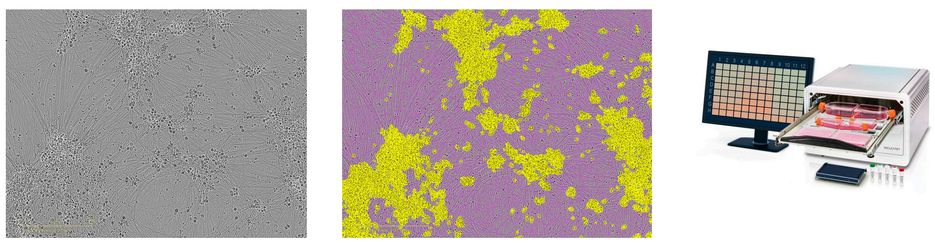

Figure 4. Masking of neuronal cell bodies (yellow) and neurite projections (pink) using the Neurotrack module on the Incucyte ® S3 platform.

Cell seeding density optimization

Seeding density was evaluated to determine optimal conditions for accurate neurite outgrowth analysis. Higher seeding densities resulted in dense neuronal networks, reducing the ability of the Neurotrack module to resolve individual neurites. An upper threshold of 100,000 cells/cm^2 was identified for reliable neurite segmentation and quantification (Figure 5, red).

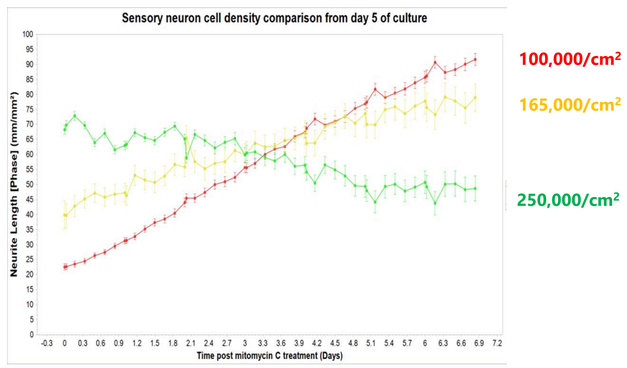

Figure 5. Evaluation of sensory neuron seeding density and the impact on effective segmentation and quantification of neurite outgrowth.

Paclitaxel-induced neurotoxicity

Paclitaxel is a neurotoxic compound that is known to inhibit neurite outgrowth. Both 1 µM and 10 µM were sufficient to inhibit neurite outgrowth. The timepoint of compound addition was optimized and showed that paclitaxel treatment initiated on day 7 of maturation produced a larger dynamic assay window at full neuronal maturity (day 21) compared with compound addition on day 14 .

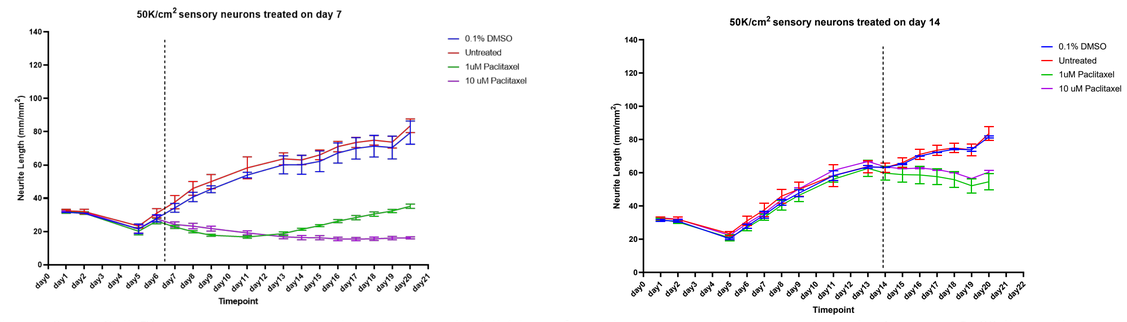

Figure 6. axoCells™ sensory neurons paclitaxel induced inhibition of neurite outgrowth measured on the Incucyte® S3 live cell imaging platform over 21 days of maturation. n=4 wells per condition.

Conclusion

Chamber-specific human iPSC-CMs show different ion channel expression and therefore pharmacological responses can differ or sometimes show opposing effects. This should be considered when running cardiotoxicity screens *in vitro*. Human iPSC-derived sensory neurons can also be used for *in vitro* toxicity assessment, as long as both seeding density and compound addition timepoint are carefully optimized to maximize the assay window at full maturity. Both models utilize multi-well plate formats and demonstrate the option of using scalable, physiologically relevant NAMs for high-throughput toxicology assessment.

References

- Lickiss B, Hunker J, Bhagwan J, et al. Chamber-specific contractile responses of atrial and ventricular hiPSC-cardiomyocytes to GPCR and ion channel targeting compounds: A microphysiological system for cardiac drug development. J Pharmacol Toxicol Methods. 2024;128:107529. doi:10.1016/j.vascn.2024.107529

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