Comparative assessment of different types of human stem cell derived cardiomyocytes for predictive electrophysiological safety screening

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Introduction

The need for new strategies in preclinical compound testing is intensively debated by the pharmaceutical industry. In this respect, cardiomyocytes generated from human stem cells (hSCs) are regarded as a promising source to develop meaningful in vitro test models of adverse cardiovascular effects, including electrophysiological safety screening. Our study is the first to compare the pharmacological profile between three types of commercially available hSCs by means of MEA recordings and to relate it to existing electrophysiological preclinical cardiac safety models.

Commercially available human embryonic or induced pluripotent stem-cell derived cardiomyocytes (hiPSCs, hESCs) from three providers were electrophysiologically validated against 28 compounds with different modes of action. Spontaneous field action potentials (fAP) were recorded from the cells directly seeded on the recording electrodes of 6-well MEAs.

Conclusion

Compound effects comprised changes of the initial phase of the fAP (Na^+ component), fAP duration as well as changes of the spontaneous beating frequency and regularity. For most of the reference compounds, all cell types investigated expressed the same alterations in the parameters analyzed in response to the compounds, but with different sensitivities. Comparison of our results with literature data from other preclinical cardiac safety models revealed in most but not all cases a good pharmacological correlation for all cell types tested.

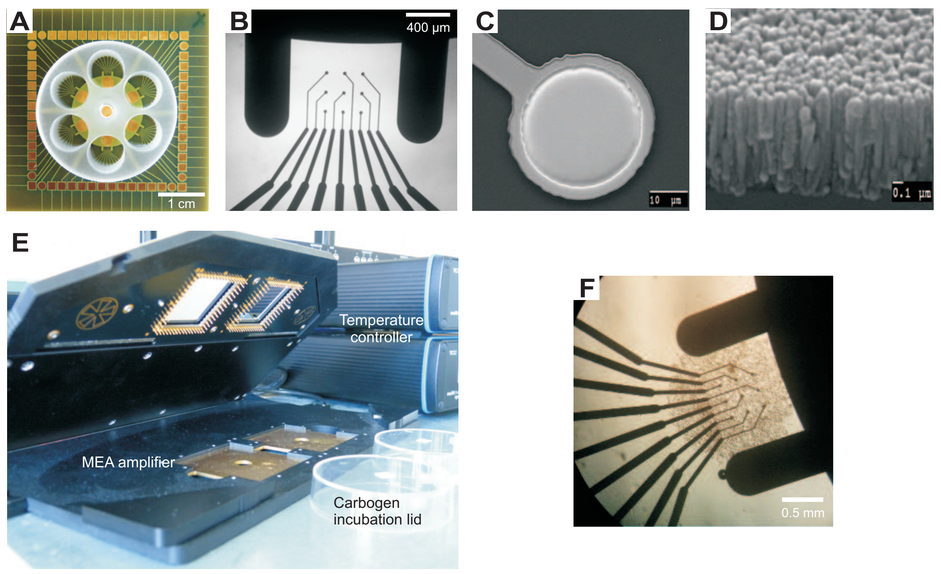
Based on these findings, we conclude that hSC-derived cardiomyocytes in principle are a promising cell source for electrophysiological cardiac safety assays but still need to be improved towards the expression of a mature electrophysiological phenotype to avoid false-negative or -positive responses.

Comparison of Predicitivty

Provider	Effect on fAP duration		Effect on amplitude		Effect on beat frequency	
	Predictions	Correct hits	Predictions	Correct hits	Predictions	Correct hits
I	28	11	24	14	21	10
II		17		14		13
III		13		12		14

Predictions: effects described in literature
Correct hits: effect as published

1. MEA Plattform & Cultivation

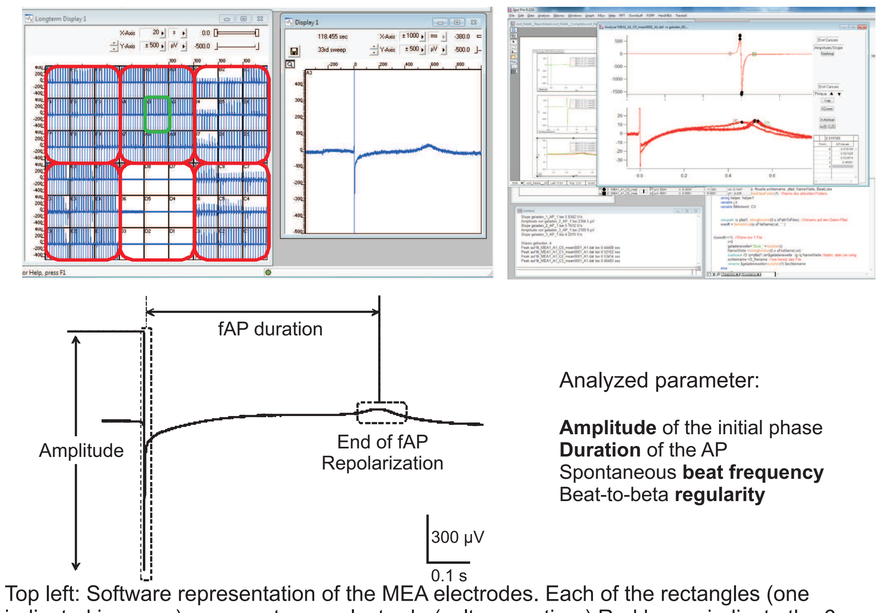


(A) The design of the 6-well Microelectrode Arrays (MEA) for extracellular recordings of electrical activity allows 6 individual experiments to be run simultaneously. **(B)** Each of the wells contains 9 recording electrodes. **(C)** Detailled view of a single TiN electrode (diameter 30 µm). **(D)** Each electrode is nanostructured providing optimal signal-to-noise ratio. **(E)** Overview of the MEA 2100 recording platform. **(F)** hSC derived cardiomyocytes seeded on the top of the electrodes in a 6-well chamber, using a special drop technique for minimized cell consumption.

2. Analysis

McRack: Recording, averaging and data export

Igor Pro: Proprietary analysis



Analyzed parameter:

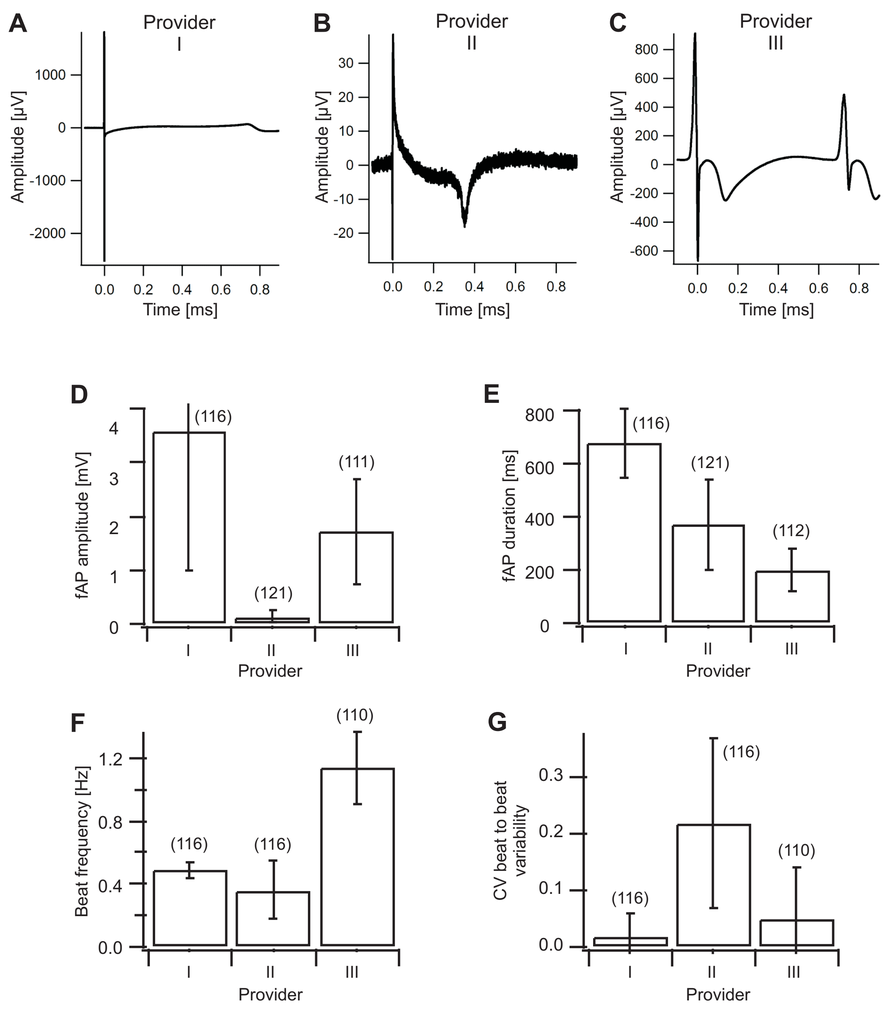
Amplitude of the initial phase
Duration of the AP
Spontaneous **beat frequency**
Beat-to-beta **regularity**

Top left: Software representation of the MEA electrodes. Each of the rectangles (one indicated in green) represents one electrode (voltage vs time).Red boxes indicate the 6 wells of the MEA. Blue traces are fAPs, here recorded in five out of the six wells. Top right: analysis is performed using self-witten macros. Bottom: overview of the most relevant fAP parameters.

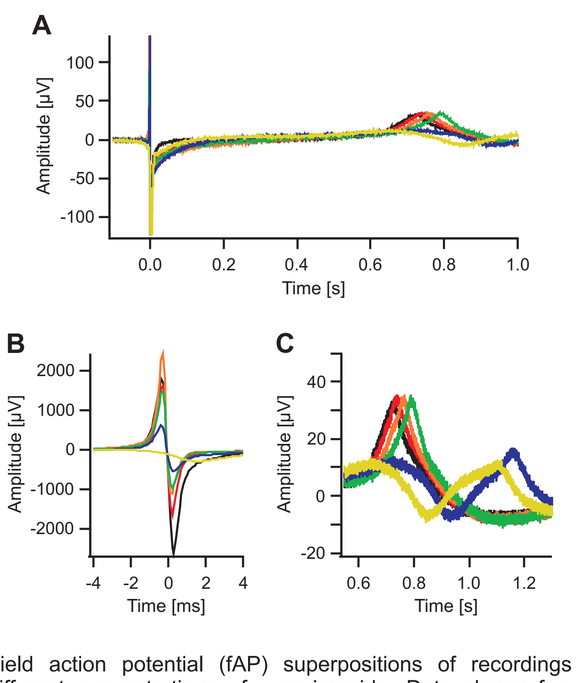
3. Compounds

Compound	Function
NS1643	hERG channel and BK-Ca channel activator
Dofetilide	hERG channel blocker
E4031	hERG blocker
Terfenadine	hERG and K-ATP channel blocker; H1 histamine receptor antagonist
Sparfloxacin	hERG channel blocker; inhibits bacterial DNA gyrase
Moxifloxacin	hERG channel blocker; inhibits bacterial DNA gyrase
Sotalol	hERG blocker; β-adrenoceptor antagonist; class III antiarrhythmic
JNJ303	I_{Ks} blocker
Levcromakalim	K-ATP channel opener
Nicorandil	K-ATP channel opener and nicotinamide nitrate NO donor
DPO-1	Kv1.5 channel (I-Kur) inhibitor
Procainamid	Na^+ channel blocker (Class Ia antiarrhythmic agent)
Quinidine	Na^+ channel blocker (Class Ia antiarrhythmic agent); hERG blocker
Mexiletine	Na^+ channel blocker (Class Ib antiarrhythmic agent), K-ATP channel opener
Lidocaine	Na+ channel blocker (Class Ib antiarrhythmic agent)
Flecainide	Na+ channel blocker (Class Ic antiarrhythmic agent)
Ranolazin	inhibits late I_{Na} and I_{Kr} currents; pFOX (partial fatty acid oxidation) inhibitor
Bepridil	non-selective calcium channel, blocks NCX and sodium channels
Diltiazem	L-type Ca^{2+} channel blocker, benzothiazepine
Bay K8644	L-type Ca^{2+}-channel activator, dihydropyridine
Nimodipine	L-type Ca^{2+} channel blocker, dihydropyridine
Verapamile	hERG blocker; L-type calcium channel blocker; α1-adrenoceptor antagonist
Ivabradine	I(f) channel blocker
SEA0400	NCX blocker
Carbenoxolone	blocks gap junction communication
Tegaserod	5-HT4 partial agonist
Carbachol	non-selective cholinergic agonist
Isoprenaline	β-adrenoceptor agonist

4. Characterization of hSC Cardiomyocytes



(**A - C)** Representative exemplary traces of field action potentials (fAP) in hSC derived cardiomyocytes fom three different commercial providers. Data were obtained under control condition. **(D - G)** Basic electrophysiological properties fAP. **(D)** average depolarizing component of the initial part of the fAPs; **(E)** fAP duration from initial phase of the fAP to the maximum of the repolarization deflection; **(F)** average beat frequency and **(G)** variability of the beat-to-beat intervals plotted as coefficient of variation (CV). Error bars represent SD, numbers in brackets indicate the number of data points.



Field action potential (fAP) superpositions of recordings at different concentrations of procainamide. Data shown from a single experiment; cells from provider I. **(A)** Overview of the fAPs in low time resolution. Voltage deflections at the beginning of the traces are truncated for displaying reasons. **(B)** time resolved beginning of the fAPs (Na^+ component) and **(C)** superposition of the repolarization phase at the end of the fAPs.
Black: control; red: 0.1 µM; orange: 1 µM; green: 10 µM; purple: 5 µM; yellow: 100 µM procainamide.

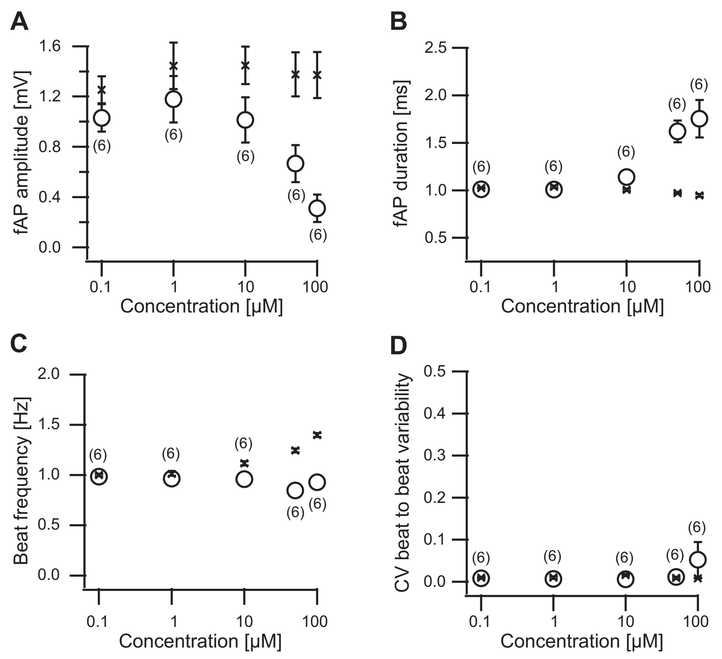
Compound Effects: fAP duration

Observed fAP duration		Expected fAP duration: ↓	Θ	↑
	Expected	10	4	14
↑	Provider I	1	1	7
	Provider II	1	1	10
	Provider III	0	1	9
Θ	Provider I	5	0	7
	Provider II	4	2	5
	Provider III	8	2	5
↓	Provider I	4	3	0
	Provider II	5	1	0
	Provider III	2	1	0

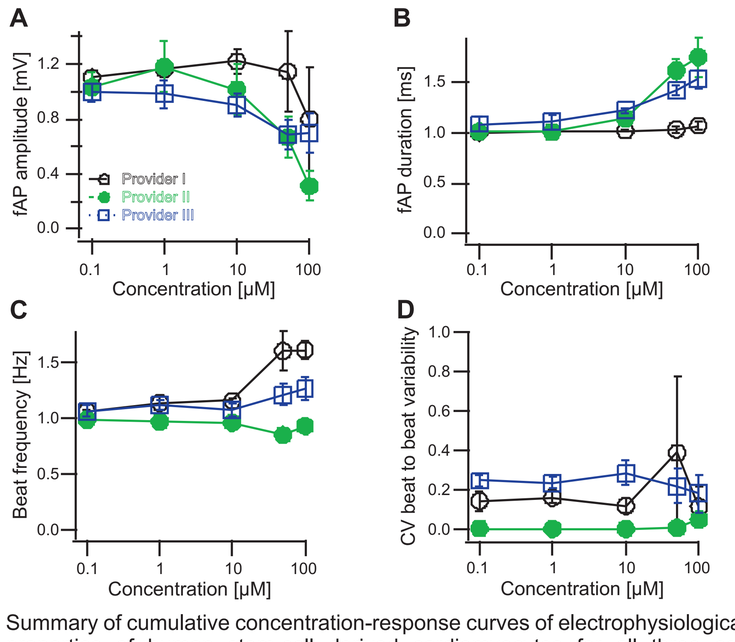
7 out of 14 compounds correctly found
7 correct hits out of 9 findings

Correlation matrix of expected vs recorded electropyhsiological effects on fAP parameters for all compounds under investigation. As example effects on the fAP duration is shown. Areas where expected and observed effects are identical are marked green.

5. Compound test



Cumulative concentration-response curves of electrophysiological properties of cardiomyocytes from provider I (open circles). **(A)** fAP amplitude of the rapid component; **(B)** fAP duration. **(C)** beat frequency. **(D)** beat-to-beat variability expressed as coefficient of variation (CV) between consecutive fAPs. Numbers in brackets indicate number of data points. Error bars represent SEM. Star data points represent time-matched solvent controls . Data from procainamide application..



Summary of cumulative concentration-response curves of electrophysiological properties of human stem-cell derived cardiomyocytes for all three cell providers. Data from procainamide application.

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