Human pluripotent stem cell-derived cardiomyocytes generated with a well-defined cardiac differentiation media kit for use in basic and applied research

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Abstract

Human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CM) provide an attractive model for development studies, disease modelling and drug discovery/safety, as well as potential source for cell therapies. For these purposes, well-defined media systems, in order to achieve highly efficient differentiations and generate large numbers of high quality cardiomyocytes, are desired. We developed a cardiac differentiation media kit that, in combination with Pluricyte® Cardiomyocyte Medium, generates predominantly ventricular cardiomyocytes with a high level of maturity. With this defined media system, we were able to achieve reproducible cardiac differentiations with high expression levels of cardiac markers. To further investigate the electrophysiology of hiPSC-CMs, these were characterized by MEA analysis using an xCELLigence RTCA CardioECR instrument. MEA analysis showed field potentials with a pronounced repolarization peak. After addition of cardioactive compounds, relevant pharmacological responses were observed.

We conclude that the cardiomyocytes generated with this cardiac differentiation media kit exhibit the required characteristics to be considered as a highly relevant model for basic and applied research studies.

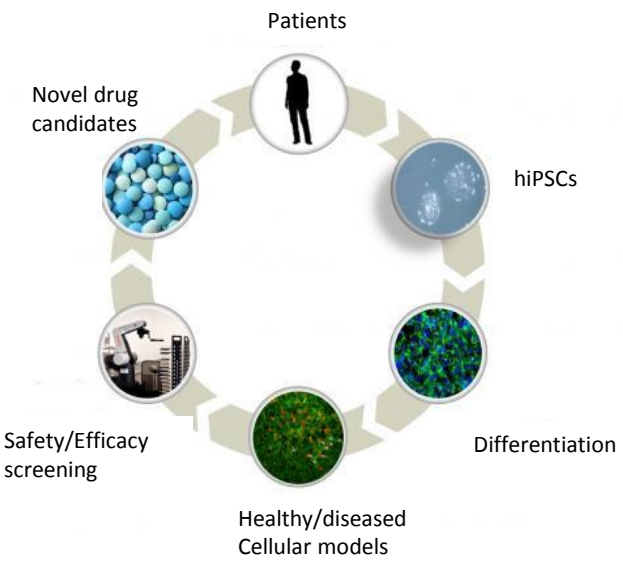

Fig. 1 Expected impact of stem cell derived cellular models in in drug discovery and development.

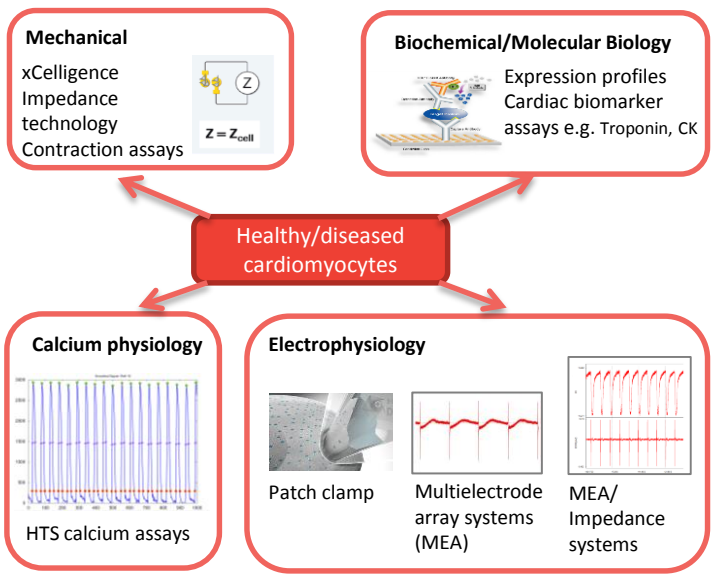

Fig. 2 Cardiomyocyte assays valuable for development studies, disease modelling and drug discovery/safety pharmacology.

Cardiac differentiation kit and procedure outline

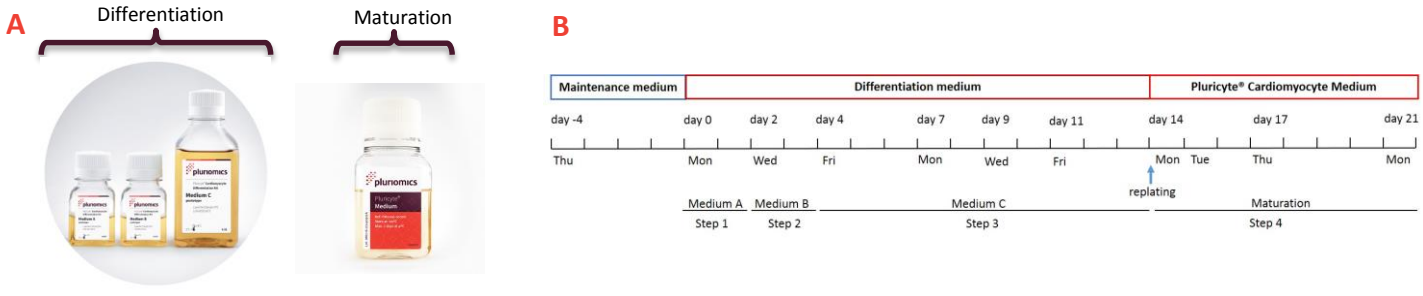

Fig. 3 (**A**) The cardiac differentiation kit consists of 3 ready-to-use serum-free medium parts (left panel). At day 14 of the differentiation procedure, Pluricyte® Cardiomyocyte Medium is used for further maturation of the generated hiPSC-CMs (right panel). (**B**) General protocol for a cardiac monolayer differentiation applicable to different hiPSC lines maintained in various culture methods. Depending on the distinct hiPSC line, contracting areas starting between day 7 to day 11.

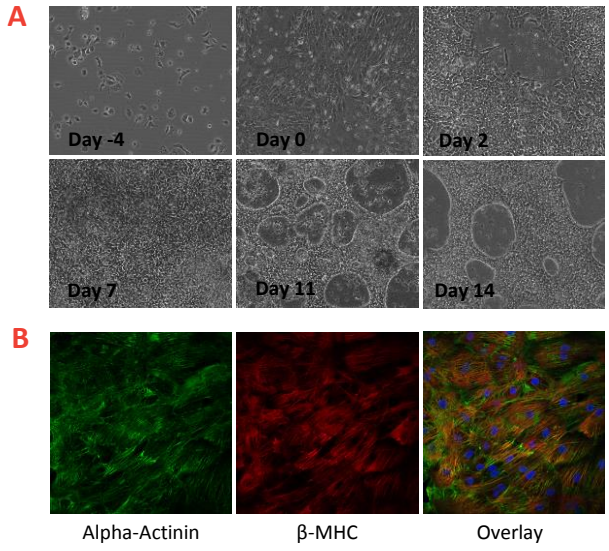

Fig. 4 Characterization of hiPSC-CMs generated by the cardiac differentiation kit. (**A**) Representative bright field images of PLM line 2 during cardiac differentiation. Contracting areas were observed at day 11. (**B**) Immunofluorescence staining of hiPSC-CMs from PLM line 1 (Green- alpha actinin, red- β-MHC, blue- nuclei) replated in Pluricyte® Cardiomyocyte Medium for 7 days. Sarcomeric structures can be observed.

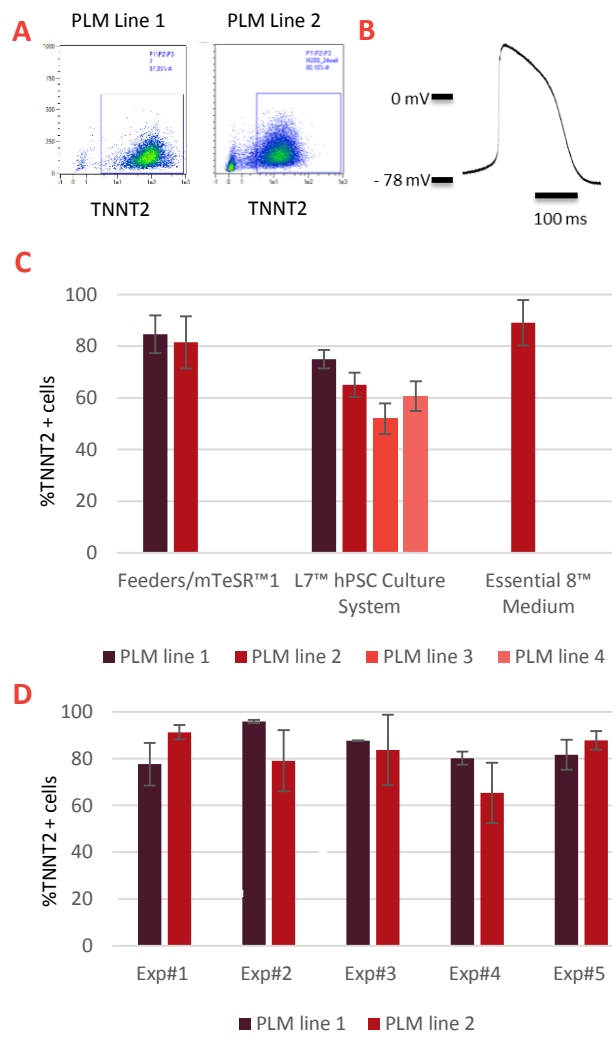

Fig. 5 Flow cytometry analysis of cardiac differentiation. (A) PLM line 1 and 2 derived cardiomyocytes, replated in Pluricyte® Cardiomyocyte Medium for 7 days, were examined for cardiac Troponin T (TNNT2) expression. (**B**) Characterisation of Pluricyte® cardiomyocytes action potential using the perforated patch-clamp technique demonstrates their predominant ventricular phenotype. **(C)** Comparison of cardiac differentiation performance across different PLM hiPSC lines based on TNNT2 expression at day 14 of differentiation maintained under feeder/mTeSR™1 (StemCell Technologies), L7™ hiPSC Culture System (Lonza) and Essential 8™ Medium (Life Technologies) as indicated. **(D)** Reproducibility of performance over 5 independent experiments. hiPSC-CMs from PLM line 1 and 2.

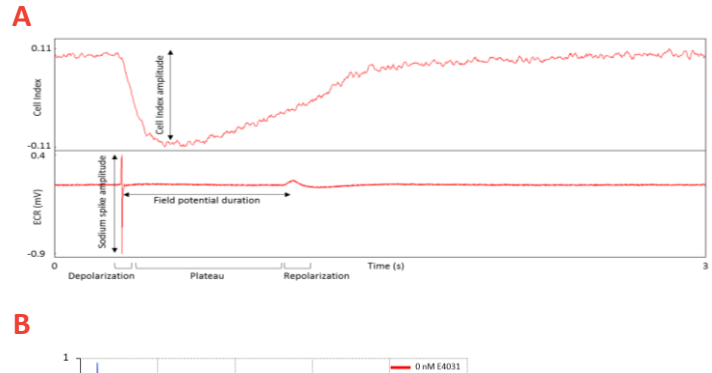

Fig. 6 Electrophysiological characterization of hiPSC-CMs, replated in Pluricyte® Cardiomyocyte Medium, using the xCelligence CardioECR (ACEA). (**A**) Parallel measurements of Cell Index (upper panel) and Field Potential (lower panel) of hiPSC-CMs from PLM line 2. The field potential shows a robust sodium spike amplitude and a well-pronounced repolarization peak. (**B**) Pharmacological response of hiPSC-CMs to E4031 which caused prolongation of field potential duration and flattening of the repolarization peak in a dose-dependent manner. At higher concentrations, arrhythmias could be observed. (**C**) Treatment of hiPSC-CMs with L-type calcium channel blocker diltiazem, which caused shortening of the field potential duration in a dose-dependent manner.

Conclusions

- We have developed an easy and ready-to-use well-defined cardiac differentiation kit that is robust and reproducible.
- Several cell lines cultured in different maintenance culture conditions have been efficiently differentiated into cardiomyocytes as shown by the expression of cardiac markers.
- Used in combination with our Pluricyte® Cardiomyocyte Medium which was previously shown to increase maturation [1], this cardiac differentiation media kit generates cardiomyocytes with a high level of maturity.
- Cardiomyocytes generated with this cardiac differentiation kit are predominantly of the ventricular subtype and exhibit the required characteristics to be considered as a highly relevant model for basic and applied research studies.

References

[1] Ribeiro MC et al. Functional maturation of human pluripotent stem cell derived cardiomyocytes in vitro-correlation between contraction force and electrophysiology. Biomaterials (2015) 51:138-50.

Acknowledgments

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