Physiological Versus Unphysiological Cell Culture Conditions: How The Environment Alters Gene Expression, Phenotype and Functional Properties of Human iPSC-Derived Cardiomyocytes

Poster #75

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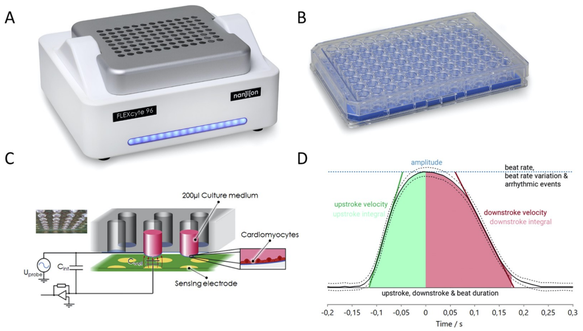
Introduction

- Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) serve as an animal-free approach to assess preclinical cardiac risk without ethical concern but high human relevance. However, their premature phenotype in standard cell culture assays including immature functional responses upon positive inotropic compound treatment, delays their full intergration in preclinical drug development.
- Here we describe the effect of the FLEXcyte technology on hiPSC-CM maturation. Expression levels of relevant cardiac genes and phenotypic characterization via actin cytoskeleton immunostainings were performed. In addition, functional characterization with cardio-safe and cardio-toxic standard compounds were performed on acute and chronic level demonstrating mature responses of hiPSC-CMs when cultured in a natural environment.
- Gene expression characterization was performed on hiPSC-CMs plated in both regular, plastic 96-well plates and FLEXcyte 96-well plates, to directly compare genotypic alterations regarding maturity elicited by a physiological versus unphysiological environment.

Methods

- The FLEXcyte technology is based on a special 96 well plate that contains ultra-thin and hyper-elastic silicone membranes instead of stiff plastic surfaces as basis for human iPSC-CMs. This FLEXcyte 96 plate is analyzed in the FLEXcyte 96 device (Fig.1A-B), an add-on system for the CardioExcyte 96 (Nanion Technologies, Munich, Germany)
- The cells adhere as monolayers on the flexible substrates. While being deflected by the weight of the culture medium, rhythmic contraction of the cardiomyocytes lifts the membranes in the 96-well plate upwards. These changes in deflection are quantified by means of capacitive distance sensing (Fig.1C).
- Parameters analyzed are contractile force (mN/mm^2), rising and falling times, AUC as well as beat duration and beat rate (Fig.1D) (Gossmann *et al.*, 2016 and 2020).

1. FLEXcyte 96 Technology



Results

2. Phenotypic characterization of hiPSC-CMs cultured on FLEXcyte 96 plates

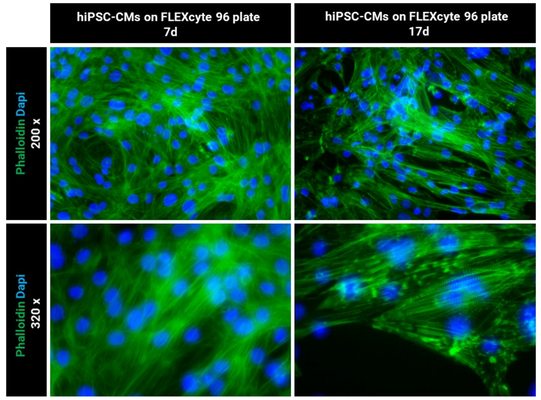


Figure 2. Phalloidin Immunostainings of hiPSC-CMs (Cardiosight-S, Nexel Ltd.) cultured on FLEXcyte 96 plates after 7 and 17 days in cuture. Cell nuclei stained with Dapi.

3. Gene expression analysis of hiPSC-CMs cultured on FLEXcyte 96 plates

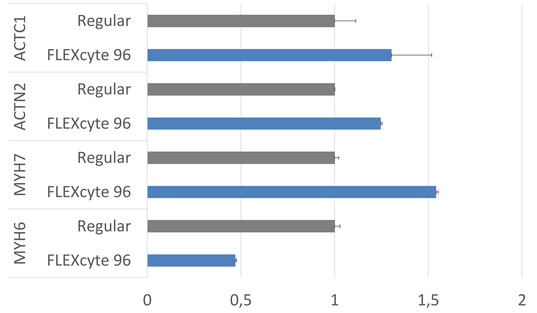


Figure 3. Gene expression analysis (MHY6, MHY7, ACTC1 and ACTN2) of hiPSC-CMs (Cardiosight-S, Nexel Ltd.) cultured on FLEXcyte 96 plates for 7 days.

4. Chronic characterization of hiPSC-CMs functional properties

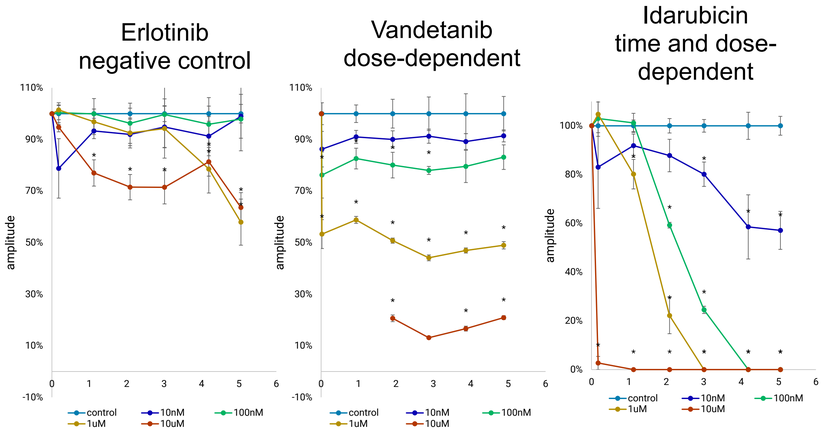


Figure 4. Time- and dose dependent effects of hiPSC-CMs (iCell CM^2, FCDI and Cardiosight-S, Nexel Ltd.) treated with TKI and anthracyclines on the FLEXcyte 96 for 5 days

Results & Conclusions

- The results show a clear pro-maturation effect on phenotypic, genetic and functional properties of hiPSC-CMs, due to the physiological environment created with the FLEXcyte 96 technology. Mature filamentous actin structures including striations at day 17 combined with the upregulated MHY7 gene, a ventricular CM specific gene, as well as upregulated alpha actin genes ACTC1 and ACTN2, demonstrate the transition of hiPSC-CMs from immature to a more mature level. On a functional level, all compounds showed the desired cardio-safe or toxic effect as known for adult cardiomyocytes. Erlotinib (cardio-safe) showed negative inotropic effects only at high (micromolar) doses, while vandetanib and idarubicin (both cardiotoxic) showed time and/or dose dependent inotropic effects.
- The displayed adult-like hiPSC-CM phenotypic, gene expression and functional properties further underline the known pro-maturation effect of the FLEXcyte 96 technology (Gossmann et al., 2020) and its potential for preclinical cardiac risk assessment.

Acknowledgements

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