A robust platform for generation and high throughput functional analysis of human iPSC-derived cardiomyocytes.

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ABSTRACT

Induced pluripotent stem cells (iPSC) derived from human patient samples can be used to generate cardiomyocytes. They hold high promise to provide more predictive and clinically relevant cell models for drug toxicity assessment and disease modeling to facilitate drug development or drug discovery pipelines respectively. Here, we describe a simple workflow that enables high-throughput functional screening with human iPSC-derived cardiomyocytes. This workflow relies on a robust system to efficiently generate cardiomyocytes from multiple human iPSC lines, yielding cardiomyocyte purities of over 50%, which can be further increased using a metabolic enrichment step. We further show that these iPSC-derived cardiomyocytes can be replated into 384-well plates to support calcium flux and membrane potential assays using Fluo-4 and FluoVolt™ dyes, respectively. We demonstrate that function of the iPSC-derived cardiomyocytes can be measured accurately as shown by the expected pharmacology and physiology of a variety of known and selective ion channel activity modulators.

INTRODUCTION

Reprogramming permits the derivation of iPSC from diseased patients, and allows us to model diseases in vitro. With the advent of CRISPR mediated genome editing, disease mutations can now also be mimicked in control iPSC lines to study the biological effect of those specific mutations. iPSC can then be differentiated into specified cell types such as cardiomyocytes and neurons which can be used to develop assays for drug safety screening or can be used to model disease phenotypes in a dish to discover new drugs[1].

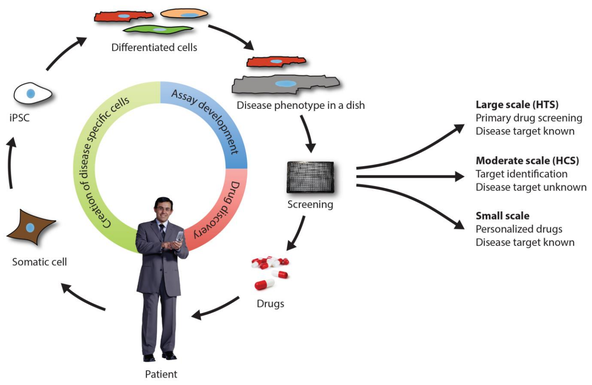


Figure 1. Overview of the applications of disease specific iPSC (and genome-edited iPSC) in the drug discovery pipeline[1].

The main challenge to implement differentiated, specialized cells as functional disease models in drug discovery is the production of cells in high purity and in large enough scale to allow small scale (typically high content image based screens, HCS) to large scale screens (high throughput, HTS) (Figure 1).
Cardiac differentiation from a variety of iPSC sources has become fairly straightforward since the publication of robust protocols, which allow generation of cultures with >90% cardiomyocyte purity[2,3]. In principal, these methods allow the generation of highly pure cultures without the need to use antibiotic resistance cassette engineered iPSC to drive purity of the cultures. However, both the antibiotic selection or high purity differentiation methods typically yield variable cardiomyocyte purity results and sometimes end up being very lengthy. Variability often originates from the biological differences between the iPSC lines (which may be caused by the different reprogramming methods being used, e.g. lentivirus, plasmid or Sendai virus based), other contributing factors include user-to-user variability or day to day media preparation differences. We therefore sought to develop a simple media system that could be used to generate cardiomyocytes from a variety of iPSC and embryonic stem cell lines that yields cardiomyocytes quickly and in a scalable fashion for producing larger amounts that can be implemented in large scale screening.

RESULTS

Our recently released Cardiac Differentiation Kit provides a ready-to-use solution to generate cardiomyocytes from human iPSC lines by a series of simple media changes, yielding highly pure populations of cardiomyocytes as early as 8 days from the start of differentiation (Figure 2).

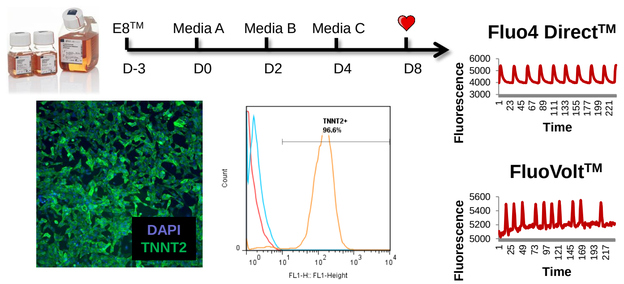


Figure 2. The Cardiomyocyte Differentiation Kit, a robust system to derive cardiomyocytes from human iPSC. High purity can be achieved as indicated by immunostaining and flow cytometry quantification of TNNT2 expressing cells. These cardiomyocytes can be furthermore used to measure cardiac function through calcium and voltage traces generated from live cell dyes Fluo4™ and FluoVolt™ respectively (right panels).

Cardiomyocytes generated by this kit can be replated and analyzed functionally using our Molecular Probes™ dyes Fluo4 Direct™ and FluoVolt™ to respectively quantitate calcium transients and membrane potentials during contraction of the cardiomyocytes (Figure 2).
To improve the robustness of the Cardiomyocyte Differentiation Kit on a variety of iPSC lines, we sought to identify the major contributors to variation between differentiation users and iPSC lines. A key factor that drives variability in cardiomyocyte purity and yield is the iPSC confluency at start of differentiation. That optimal confluency was found to vary widely between different cell lines (Figure 3); however, once that optimal confluency for a particular iPSC line was identified, reproducible results were maintained between runs and users as shown. Regardless of optimal confluency, in some iPSC lines the increasing number of passages post-thaw appeared to negatively affect the ability of those iPSCs to differentiate to the cardiac lineage (not shown), which could be prevented by simply starting each differentiation from a newly recovered frozen vial of iPSCs.

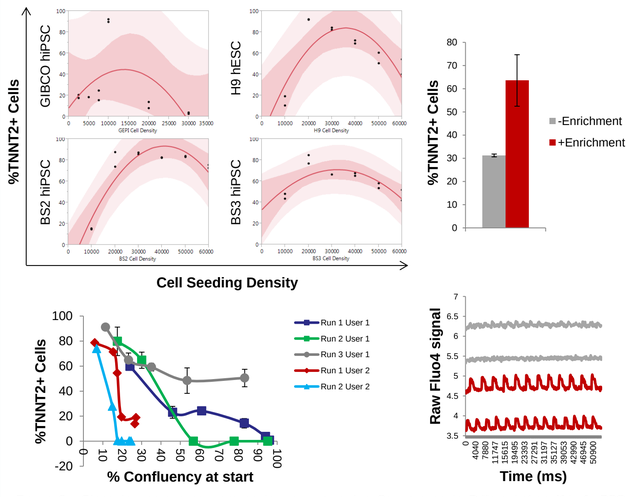


Figure 3. Steps to improve cardiomyocyte yield using the Cardiomyocyte Differentiation Kit. A iPSC seeding density range study should be performed for each iPSC line as indicated, once those conditions have been identified, consistency can be maintained between users and runs (top and bottom left). For iPSC lines that still do not provide robust cardiomyocyte purity, a metabolic enrichment protocol can be added at the end of the differentiation protocol (top and bottom right).

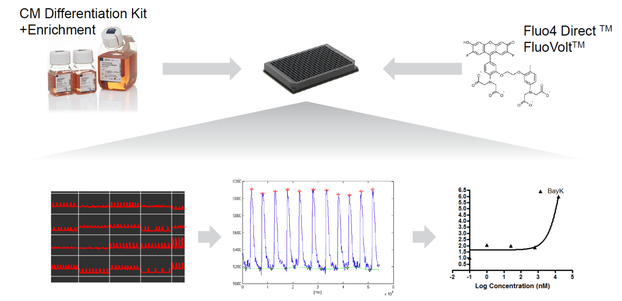
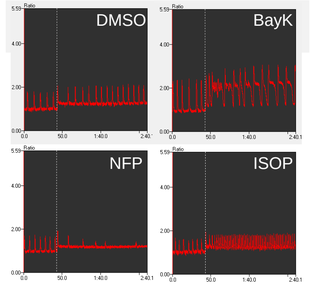


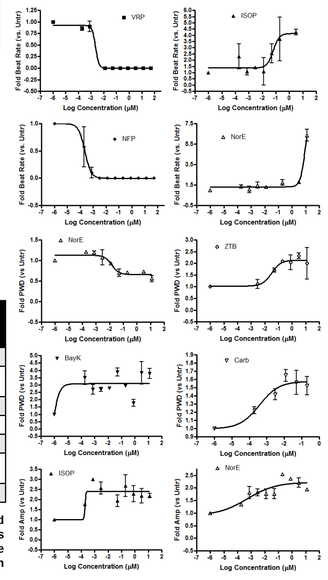
Figure 4: Overview of a robust system for generation and high throughput functional analysis of hiPSC-derived cardiomyocytes. Applications include drug safety testing and drug discovery in cardiac disease models.

While the cardiac differentiation kit was developed to yield high purity cardiomyocyte preps from the majority of iPSC lines, there may be lines that that differentiate into cardiomyocytes with lower efficiency. For those lines, a metabolic selection step, which relies on the use of lactate instead of glucose, was tested (Figure 3). Under low cardiac differentiation yield circumstances, cultures could be enriched to 50-80% purity, which was sufficient to run functional cardiomyocyte assays (Figure 3).
These kit-generated iPSC-derived cardiomyocytes were used in functional high throughput assays for in vitro modeling of cardiac diseases or for cardiac safety testing of drug leads, as the kit-generated cardiomyocyte cultures can be dissociated and seeded into multiwell plates at about 10-14 days from the start of differentiation. The cardiomyocytes can then be loaded with Fluo4 Direct™ or FluoVolt™ and assayed in up to 384 wells simultaneously on a Hamamatsu FDSS6000 instrument (Figure 4).



Compound	Beat Rate EC_{50}/IC_{50} (nM)	Beat Amp EC_{50}/IC_{50} (nM)	Beat Length EC_{50}/IC_{50} (nM)
Verapamil (VRP)	2.3	26	9
Isoproterenol (ISOP)	63	0.2	0.2
BayK 8644	N/A	0.2	0.0004
Carbachol (Carb)	N/A	N/A	0.4
Nifedipine (NFP)	0.2	1.1	1.7
Norepinephrine (NorE)	83	0.5	14
Zatebradine (ZTB)	N/A	N/A	32

Figure 5. Pharmacokinetic responses on iPSC-derived cardiomyocytes using Fluo4 Direct™. Calcium traces during compound treatment and the derived dose response curves with EC_{50}/IC_{50} values are shown above and to the right respectively.



This system was tested with a panel of well-known agonists and antagonists that target cardiomyocyte function (Figure 5). Representative calcium traces during treatment with DMSO, Isoproterenol (ISO), Nifedipine (NFP) and BayK 8644 (BayK) are shown in Figure 5 (white dotted lines indicate time of small molecule addition). From the calcium or voltage traces, different parameters can be easily extracted to visualize effects of drugs on cardiomyocytes and the following three are presented here: beat rate, beat duration or pulse width duration (PWD) and beat intensity or amplitude (AMP). Representative dose response curves for these parameters obtained with Fluo4 Direct™ on iPSC-derived cardiomyocytes are shown for well known ion channel agonists and antagonists (Figure 5). From these curves IC_{50}/EC_{50} values can be obtained for the well known ion channel agonists and antagonists and were found to be in line with published reports on these drugs in a variety of cardiomyocyte assays.

CONCLUSIONS

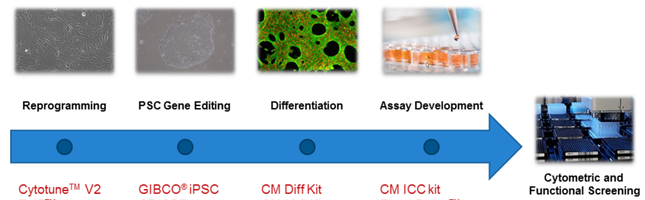


Figure 6. Overview of the cardiomyocyte differentiation workflow for disease modeling and drug safety testing, ranging from reprogramming over genome editing to differentiation and high throughput assays. Products supporting the entire workflow are indicated in red.

Implementation of human iPSC-derived cardiomyocytes for toxicity and drug discovery screening has been steadily increasing in academic and pharmaceutical pipelines. Here we demonstrated that a simple kit can be used for reliable and consistent generation of cardiomyocytes from a variety of iPSC lines for functional cardiomyocyte analysis at high throughput scale. We concluded that this system is very robust between users and cell lines when optimized properly. When needed, a lactate enrichment step to drive cardiomyocyte purity can be added on for iPSC lines that are difficult to differentiate to cardiomyocytes (www.thermofisher.com/cardiacdiff).
Biologically and functionally, we have found that these cardiomyocytes respond pharmacologically to well known heart affecting agonists and antagonists and expect that cardiac specific mutations that have functional effects can be equally measured as a readout of the disease phenotype. In addition to what we are presenting here, these cardiomyocytes can be equally implemented for HCS studies, such as hypertrophy models or cardiomyocyte metabolic health and viability.
With inclusion of the Cardiomyocyte Differentiation Kit we thus provide the tools for the entire workflow in the iPSC-derived cardiomyocyte space to develop in vitro disease models or safety testing platforms, which can be started from diseased fibroblasts or peripheral blood monocytes through reprogramming or CRISPR/Cas9 edited iPSC (Figure 6).

REFERENCES

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2. Burridge et al. Production of De Novo Cardiomyocytes: Human Pluripotent Stem Cell Differentiation and Direct Reprogramming. Cell Stem Cell 2012.
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TRADEMARKS/LICENSING

www.thermofisher.com/cardiacdiff