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Human Pluripotent Stem Cell-Derived Cardiomyocytes: Maturity and Electrophysiology

Ville Kujala, Mari Pekkanen-Mattila and Katriina Aalto-Setälä
University of Tampere, Institute of Biomedical Technology, Tampere, Finland

1. Introduction

Human pluripotent stem cells comprise embryonic and induced pluripotent stem cells. Both of these are able to give rise to all cell types of an individual, including heart muscle cells or cardiomyocytes. First stable human embryonic stem cell (hESC) lines were derived in 1998 by James A. Thomson and his co-workers (Thomson et al., 1998). First cardiomyocytes derived from hESCs were made in 2001 by Kehat and co-workers (Kehat et al., 2001) and after that many research groups have derived and studied human pluripotent stem cell derived cardiac cells and their properties. In 2007 the first human induced pluripotent stem cells (hiPSCs) were produced by Shinya Yamanaka's (Takahashi et al., 2007) and James A. Thomson's (Yu et al., 2007) groups from dermal fibroblasts, and hiPSCs have subsequently also been shown to be able to give rise to cardiomyocytes (Zhang et al., 2009, Zwi et al., 2009). Pluripotent stem cell derived cardiac cells have a great potential for cardiotoxicity testing, for preclinical testing of new chemical entities for the pharmaceutical industry and hopefully in the future for cell therapy in myocardial infarction and heart failure. However, before these goals are achieved thorough characterization of the cardiomyocytes is needed to ensure their feasibility for these applications.

2. Differentiation of cardiomyocytes from pluripotent stem cells

Cardiomyocytes can be differentiated from pluripotent stem cells by multiple methods and the differentiation event is quite rapid, 10-20 days. However, all the differentiation methods have common problems, which include uncontrolled differentiation, low differentiation rate and heterogeneous differentiated cell population. In addition, the cardiomyocyte differentiation efficiency has been shown to vary markedly between different hESC lines (Pekkanen-Mattila et al., 2009). The differentiation methods are described in more detailed manner below and summarized in Figure 1.

2.1 Spontaneous differentiation in embryoid bodies

Cardiomyocyte differentiation from hESCs and hiPSCs in EBs has been described in many reports (Figure 1 A) (Itskovitz-Eldor et al., 2000, Kehat et al., 2001, Burridge et al., 2007, Zhang et al., 2009). In addition to cardiomyocyte generation, EB differentiation is widely

used also in production of other cell types such as neuronal cells, hematopoietic cells, adipocytes and chondrocytes (Pera & Trounson, 2004). For the whole existence of hESCs, EB differentiation has been widely used differentiation method for its relatively simple and inexpensive nature regardless the low differentiation rate. For example, if aiming at cardiac differentiation, under 10% of the EBs formed contain beating areas (Kehat et al., 2001).

2.2 Differentiation with mouse visceral endoderm –like cells

A little more directed way to differentiate cardiomyocytes from hESCs is in co-culture with mouse endodermal-like (END-2) cells (Figure 1B) (Mummery et al., 2003, Passier et al., 2005). The differentiation inducing factors are secreted from END-2 cells and therefore END-2 conditioned medium can also be used in cardiomyocyte differentiation (Graichen et al., 2008). With END-2 methods, cardiogenic differentiation potential can be enhanced with serum-free medium supplemented with ascorbic acid (Passier et al., 2005) or adding cyclosporine A to the culture medium (Fujiwara et al., 2011).

END-2 cells support the differentiation towards endodermal and mesodermal derivatives (Mummery et al., 2003, Passier et al., 2005, Beqqali et al., 2006). This is in accordance with embryonal development studies, which have shown that anterior visceral endoderm is essential in normal heart development (Lough & Sugi, 2000). The specific mechanism or the specific factors inducing cardiac differentiation by END-2 cells are, however, not clearly known. It has been suggested that removal of insulin by END-2 cells could have a role in this differentiation method. Insulin inhibits cardiac differentiation by suppressing endoderm and mesoderm formation and favouring ectoderm differentiation (Freund et al., 2008) therefore the elimination of insulin from the medium by END-2 cells could favour the cardiac differentiation. Additionally, another more promising mechanism inducing cardiac differentiation by END-2 cells has been suggested to be prostaglandin I2 (PGI2) (Xu et al., 2008).

2.3 Cardiac differentiation with defined growth factors

The combination of activin A and BMP-4 with matrigel has been used in differentiation protocols for cardiomyocytes (Figure 1C) (Laflamme et al., 2007). These factors enhance mesoendoderm formation, an early precursor cell lineage which gives rise to mesoderm and endoderm (Laflamme et al., 2007). Mesoderm is the origin of cardiac cells , but cardiac differentiation inducing signals are in large extent arising from endoderm (Lough & Sugi, 2000).

A stepwise differentiation protocol has also been developed by Gordon Keller's group (Yang et al., 2008, Kattman et al., 2011). This protocol involves induction of primitive streak-like population, in addition to formation of cardiac mesoderm and expansion of cardiac lineages. The protocol is based on EB differentiation and is comprised of three stages. Growth factors BMP-4, FGF, activin A, vascular endothelial growth factor (VEGF) and dickkoptf homolog 1 (DKK1) were used in varying combinations. Mesoendoderm formation has also been induced by Wnt3A, an activator of the canonical Wnt/β-catenin signalling pathway (Tran et al., 2009).

3. Characteristics of differentiated cardiomyocytes

Cardiac differentiation can be followed by multiple markers at gene and protein expression levels. During early stages of differentiation mesoderm formation is detectable by the elevated mRNA level of Brachyury T. Brachyury T expression peak is detected at day 3 in

END-2 co-cultures (Beqqali et al., 2006, Pekkanen-Mattila et al., 2009) and a day later in EBs (Bettiol et al., 2007, Pekkanen-Mattila et al., 2010). The cardiac differentiation cascade can be

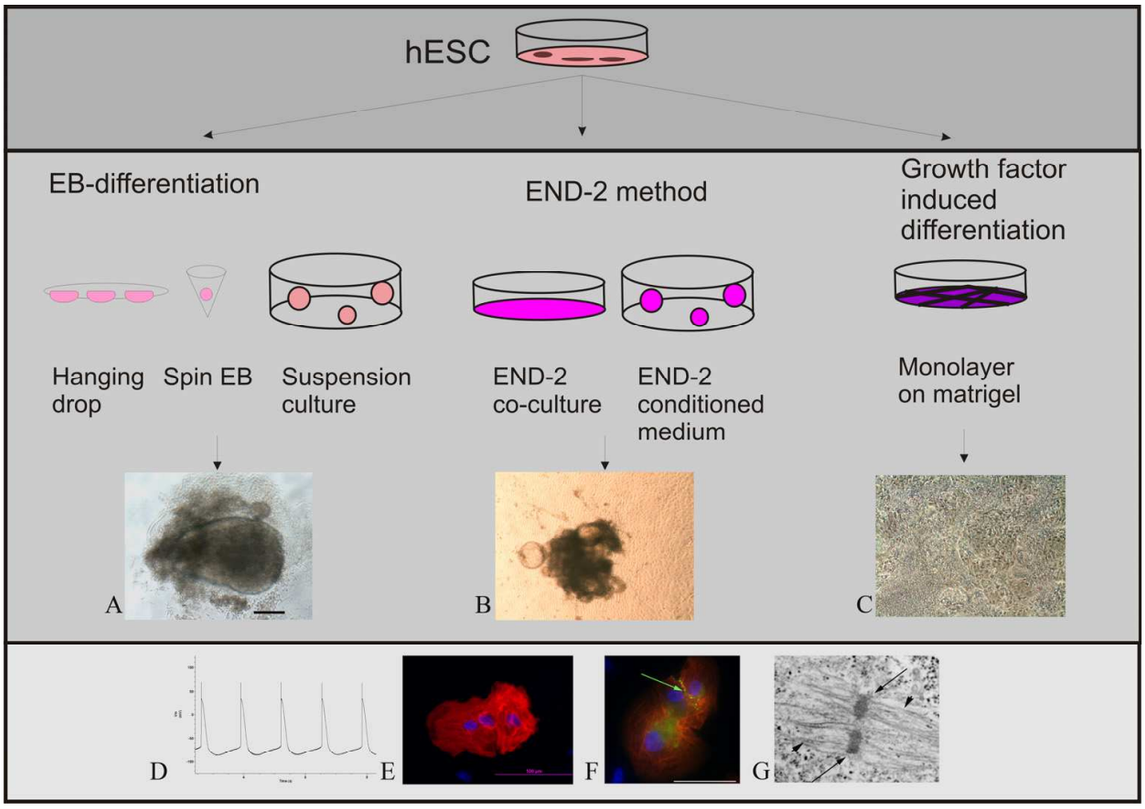


Fig. 1. Differentiation methods for hESC derived cardiomyocytes (middle column) and characteristics of the differentiated cells (undermost column). *EB-differentiation* can be performed in three ways, EBs can be formed either in hanging drops: enzymatically dissociated stem cells are pipetted in suspension into small drops on petri dish which is then inverted. EBs form in these drops and they can be plated down afterwards. EBs can be formed also in 96-well-plate wells, where single cell suspension is added and EB formation is forced by sentrifuging. Traditional was to EB formation is however the suspension method, where EBs form spontaneously in suspension from enzymatically dissociated hESCs. The picture A represents a 12-day old EB is attached to the bottom of cell culture dish.
Differentiation with END-2-cells can be performed in two ways; hESC are plated onto END-2 cell layer and differentiated as co-culture or hESC are differentiated as EBs in END-2 conditioned medium. The picture B is taken from END-2 co-culture after one week of differentiation. Third way presented here is to culture hESCs on matrigel and initiate differentiation towrads cardiac lineage with *growth factors*, such as Activin-A and BMP-4. Differentiation is performed in a monolayer of cells (C).
The first sign for differentiated cardiomyocytes is the formation of beating areas. hESC-CM have the ability to contract spontaneously and in addition to fire spontaneously action potentials (D). Differentiated cells are stained positively with troponin T (E) and connexin 45 can be seen in the border areas of two troponin T positive cells (F). Electronmicroscopy (G) reveals sarcomere structures with Z-bands (marked with arrows) in the differentiated hESC-CMs.

followed further by the expression of the cardiac regulatory transcription factors such as Islet-1 (ISL-1), Mesp 1, GATA-4, NKX2.5 and Tbx6 (Graichen et al., 2008, Yang et al., 2008).
The first clear indication for emergence of cardiomyocytes is the formation of spontaneously beating aggregates or areas in the cell culture dish (Kehat et al., 2001, Mummery et al., 2003). The number of beating areas has been used in quantifying differentiation efficiency even though the beating areas contain varying numbers of cardiomyocytes (Passier et al., 2005, Pekkanen-Mattila et al., 2009, Pekkanen-Mattila et al., 2010). The amount of cardiomyocytes has also been quantitated using flow cytometry (Kattman et al., 2011). Electron microscopy studies reveal that the differentiated cardiomyocytes contain myofibrils which are first organized randomly throughout the cytoplasm. However, organized sarcomeric structures occur at later stages of differentiation with A, I, and Z bands (Figure 1G). In the vicinity of the sarcomeres, mitochondria are also present. In addition, cells have intercalated disks with gap junctions and desmosomes (Kehat et al., 2001, Snir et al., 2003, Pekkanen-Mattila et al., 2009). Cardiac structural proteins such as troponin I, T or C (Figure 1 E), myosins and α-actinin are also present in differentiated beating cells (Kehat et al., 2001, Mummery et al., 2003).
The beating function of heart muscle is the result of chain reactions between many ionic currents through cell membranes and sarcomeric proteins in the cytoplasm. Cardiomyocytes express ion channels and gap junction proteins on the cell membrane and these proteins are needed for transmission of electrical stimuli from cell to another (Figure 1 F) (He et al., 2003, Sartiani et al., 2007). Furthermore, there are intracellular channels such as ryanodine receptor 2 (RyR2) which are responsible for the calcium-induced calcium release (CICR) from the sarcoplasmic reticulum (Fabiato, 1983, Dolnikov et al., 2006, Satin et al., 2008). All these channels function in a cascade which results in synchronous contraction of the heart mediated by sarcoplasmic proteins.

4. Cardiomyocytes during embryonic development and stem cell differentiation

The development of the heart is composed of series of complicated differentiation events and morphogenetic changes (Buckingham et al., 2005). Additionally, electrical activities of cardiac cell change during development. For example, the average beating rate of human neonatal cardiomyocytes is ~140 beats per minute and in adult cardiomyocytes ~80 beats per minute (Huang et al., 2007). The data of human cardiac embryology is, however, very restricted and, thus, most information is based on animal models. Microelectrode recordings from chick embryo revealed that the first contracting cardiomyocytes have pacemaker like action potentials. According to these measurements, the membrane diastolic potential in the first pacemaker like cells is in the range of -35 mV, action potential amplitude is relatively small and Ca^{2+} dependent action potential upstroke is slow (Sperelakis & Shigenobu, 1972).
Similar postnatal development changes are seen in rabbit models. The beating rate decreases, action potential duration increases and the maximal diastolic potential (MDP) reaches more negative values during development (Toda, 1980). Allah and co-workers investigated in rabbits mRNA levels of several ion channels and Ca^{2+} handling proteins such as hyperpolarization activated cyclic nucleotide-gated potassium channel 4 (HCN4), $Na_{V1.5}$, $Ca_{V1.3}$, NCX 1, $K_{V1.5}$, ERG, K_VLQT1 (also known as KCNQ1) and minK and they observed decreased mRNA levels of all these factors and suggested that this could explain the postnatal decrease in the beating rate and increase of action potential duration (Allah et al., 2011).

Ca-handling in cardiomyocytes also changes during embryonic development. According to ultrastructural studies, the sarcoplasmic reticulum is not completely developed at the early developmental stages and therefore neonatal cardiomyocytes are suggested to be more dependent on transsarcolemmal Ca^{2+} influx than sarcoplasmic Ca^{2+} release (Brook et al., 1983, Nakanishi et al., 1987, Nassar et al., 1987, Klitzner & Friedman, 1989). Therefore Na^{+}/Ca^{2+} exchanger (NCX) have been suggested to have a important role in excitation-contraction coupling during the early developmental stages (Klitzner et al., 1991, Wetzel et al., 1991, Wetzel et al., 1991, Huynh et al., 1992) . Indeed, NCX gene and protein expression, in addition to NCX current have been shown to be enhanced at the early developmental stages (Artman, 1992, Artman et al., 1995, Chin et al., 1997, Haddock et al., 1997, Gershome et al., 2011).

Asp and colleagues compared the cardiac marker and ion channel expression of human embryonic stem cell derived cardiomyocyte (hESC-CM) clusters to human fetal, neonatal and adult atrial and ventricular origin heart tissue samples (Asp et al., 2010). They found the beating frequencies between hESC-CM clusters to vary substantially. They could identify two groups, one having slow (< 50 beats per minute[bpm]) and the other high (> 50 bmp) beating rate and they suggested these could represent ventricular and atrial type of cardiac cells, respectively. They also demonstrated that hESC-CMs had higher *NKX2.5* and cardiac muscle actin mRNA expression levels than the heart samples. The *NKX2.5* expression level, however, decreased over time in culture finally approaching that of the heart samples. Cardiac troponin T was more strongly expressed in ventricular samples and in hESC-CMs compared to atrial samples. The levels of cardiac troponin T mRNA also decreased over time in hESC-CMs. Phospholamban was expressed less in the atrial samples and hESC-CMs compared to ventricle samples. α-myosin heavy chain, which is normally mostly expressed in atrial tissue, was more strongly expressed in hESC-CMs with beat rates over 50 bpm and in adult atrial tissue samples whereas β-myosin heavy chain was more expressed in the ventricular heart samples and less in all the other heart preparations of hESC-CMs. The α-myosin heavy chain mRNA levels increased with increasing age of the hESC-CMs. Across all the heart tissue and hESC-CM samples cardiac RyR2, L-type calcium channel, and $Na_{V1.5}$ sodium channel mRNA were similarly expressed. HERG mRNA expression in hESC-CMs was similar to neonatal and adult atrial samples. *HCN2* was expressed in a more comparable way to the ventricular samples. Only *HCN4* expression differed between the hESC-CMs, with those having the beating rate of less than 50 bpm having lower expression. Overall, it was concluded that the difference between slow beating and fast beating hESC-CMs paralleled the human atrial and ventricular tissues (Asp et al., 2010). Despite small differences in expression levels, the conclusion from this study was that stem cell -derived cardiac cells share many similarities with human heart tissue and thus stem cell -derived cardiac cells are a good cellular model for human heart.

5. Electrophysiology of human pluripotent stem cell derived cardiomyocytes

The electrical properties of pluripotent stem cell derived cardiac cells have been studied principally either with patch clamp analysis of single cells or with microelectrode array (MEA) platform using beating cell aggregates.

5.1 Patch clamp analysis

Patch clamp method has been developed to study ion channels in excitable membranes. (Sakmann & Neher, 1984). In this technique micropipette is attached to the cell membrane

by a giga seal and this can be exploited to measure current changes and voltage across the membrane. This technique has been widely used in detailed electrical analysis of pluripotent stem cell derived cardiomyocytes.

Key cardiac ion channel types (and respective currents in brackets) involved in the human ventricular action potential include $Na_{V1.5}$ (I_{Na}), $K_{V4.3}$ (I_{to}), $Ca_{V1.2}$ ($I_{Ca,L}$) $K_{V11.1}$ (I_{Kr}), $K_{V7.1}$ (I_{Ks}), and $K_{ir2.X}$ (I_{K1}) (Pollard et al., 2010). These ion channels mediate the complex interaction between the currents and result in the characteristic action potential shape which can be divided into five different phases (Figure 2). Phase 0 of the action potential is the depolarization of the cardiomyocytes from the negative membrane potential to positive, called the upstroke. This is followed by phase 1, the short transient repolarization that is followed by phase 2, the plateau at slightly less positive membrane potential than the

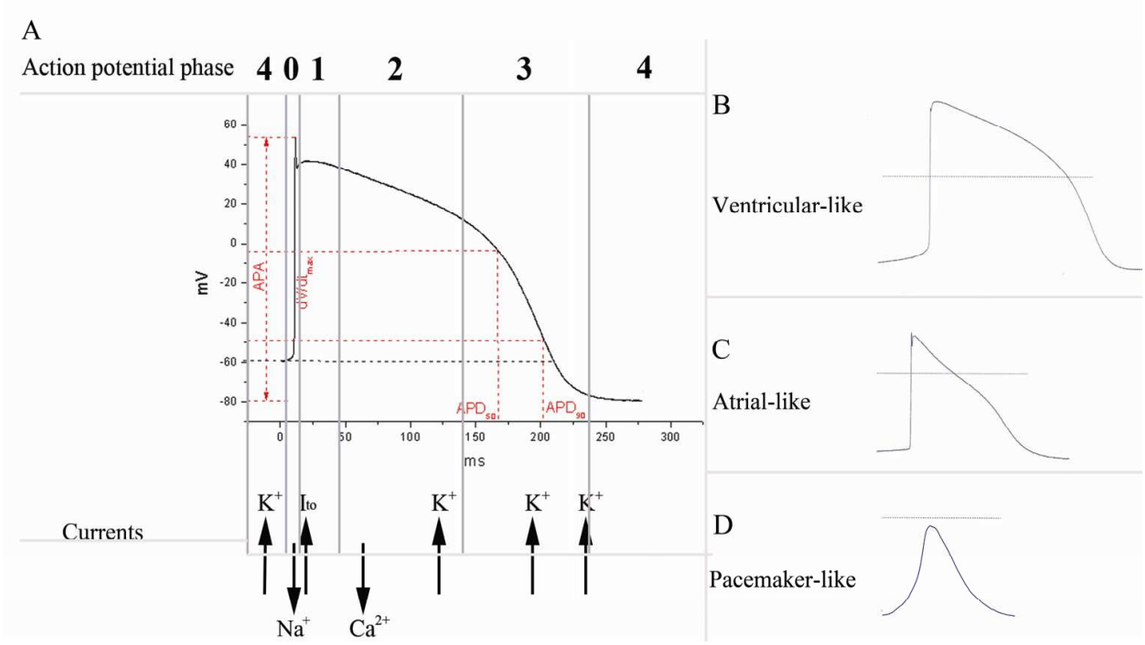


Fig. 2. Action potential phases and specification of cardiomyocyte subtypes. (A) Action potential (AP) parameters: Action potential amplitude (APA), maximum rate of rise of the action potential (dV/dtmax), action potential delay (ADP) and membrane diastolic potential (MDP). AP phase 0 is a rapid depolarization phase when the sodium channels are activated and membrane permeability is increased to Na+. Rapid depolarisation is followed by rapid repolarization phase 1 and plateau phase 2, where Ca2+ ions are entered to the cell throught L-type calcium channels. At phase 3, calcium channels are inactivated and repolarization is caused by outward potassium currents. Repolarization is due to the currents carried mainly by the slow Iks and rapid Ikr components of the delayed rectifier potassium channels. The Ikr current is produced by hERG channel (encoded by the human ether-à-go-go-related gene). By contrast, inward potassium current contributes to the maintenance of the resting membrane potential, phase 4. B-D. Classification of ventricular (B), atrial (C) an pacemaker-like (D) action potentials. Ventricular action potential has a prominent plateau phase whereas atrial action potential is more triangularly shaped. Pacemaker-like cells are characterized by slower upstroke velocity and amplitude if compared to ventricular and atrial type of cells.

maximal upstroke value. Phase 2 is followed by phase 3, which is the repolarization back to the resting membrane potential. The resting state of the membrane potential is phase 4 (Nerbonne & Kass, 2005).

Sartiani and co-workers have investigated the expression of different ion channel proteins and respective ion currents with patch clamp technique in undifferentiated hESCs and in their early- (days 15 to 40) and late-stage (days 50-110) cardiomyocyte derivates using the EB differentiation method (Sartiani et al., 2007). Some ion currents were present readily in the undifferentiated cells (I_{Kr}, I_f, $I_{ca,L}$). The properties of these currents were modified during hESC-CM development and some new currents (I_{to} and I_{K1}) were introduced, so the cardiomyocytes achieved maturation over time. With regard to the I_{to}, transient outward potassium current, the two isoforms $K_{v1.4}$ and $K_{v4.3}$ were differentially expressed in the developing cardiomyocytes at mRNA level. The shorter $K_{v4.3}$ splice variant was expressed in over 57-days-old cardiomyocytes whereas the longer one was expressed in the earlier cardiomyocytes. The $K_{v1.4}$ isoform is expressed at least from day 25 onward. Despite $K_{v4.3}$ mRNA expression in hESCs I_{to} current was not present in them. I_{to} could be detected using patch clamp on day 12 in the developing hESC-CMs and it was higher in later-stage (57 days old) cardiomyocytes compared to earlier ones (Sartiani et al., 2007).

Another repolarizing current (I_{Kr}), encoded by the human ether-a-go-go related gene (*HERG*) channel is also expressed in hESCs as well as the developing cardiomyocytes. However, the shorter splice variant *HERG1b* mRNA was only expressed in the developing hESC-CMs. Using patch clamp an outward K^+-current sensitive to E-4031, a selective blocker of the I_{Kr} current, could also be recorded in hESCs. *HCN* isoforms encode the I_f depolarization current. *HCN1* and *HCN4* were expressed more strongly in the undifferentiated hESCs and early cardiomyocytes than in late cardiomyocytes. *HCN4* was also expressed in the adult heart, whereas *HCN1* was not. *HCN2* was expressed in the adult heart as well as strongly in hESCs and early and late hESC-CMs. Voltage clamp experiments revealed I_f currents in hESCs and early and late developing cardiomyocytes. However, during cardiomyocyte maturation, the I_f activation rate decreased. With regard to another depolarization current $I_{K1,}$ the $K_{ir2.1}$ mRNA was already present in hESCs but the current could only be measured from the developing cardiomyocytes (Sartiani et al., 2007).

Voltage-dependent Ca^{2+} current ($I_{Ca,L}$) is mediated by α1C subunit of the calcium channel in many tissues and this subunit is encoded by *CACNA1C* gene. Sartiani and co-workers demonstrated mRNA expression of *CACNA1C* to be present both in undifferentiated hESCs and in the cardiomyocytes. Also, the $I_{Ca,L}$ current could be recorded from hESCs as well as the hESC-CMs. During hESC-CM development the action potential upstroke velocity and action potential duration increased significantly. The beating rate on the other hand decreased during cardiomyocyte maturation and the diastolic depolarization rate flattened in the late cardiomyocytes. Pharmacological interventions also demonstrated intact ion channel function and expected responses were obtained with E4031 and $BsCl_2$ (I_{K1} blockers), zatebradine (I_f blocker), and lacidipine ($I_{Ca,L}$ blocker). Finally, stimulation with isoprenaline proved intact β-adrenergic signalling in the stem cell derived cardiomyocyte (Sartiani et al., 2007).

Taken together, the cardiomyocytes seem to achieve a more mature cardiac phenotype over time in cell culture, even though this has not been confirmed in all studies (Pekkanen-Mattila et al., 2010). The I_{to} and I_{K1} currents could serve as markers for hESC cardiac differentiation (Sartiani et al., 2007) since they appear only later in cardiac differentiation. I_{to} current has also been shown to increase in postnatal rat cardiomyocytes (Guo et al., 1996,

Shimoni et al., 1997) and I_{K1} current has been shown to stabilize the diastolic potential in myocytes (Silva & Rudy, 2003).

With regard to the EB and END-2 co-culture differentiation methods our own experiments demonstrated that the EB method produces slightly more cardiomyocytes with consistent beating rate and more cardiomyocytes with ventricular type action potentials. The EB method also produced cardiomyocytes with significantly more hyperpolarized MDP (Pekkanen-Mattila et al., 2010). Low expression of I_{K1} current in developing hESC-CMs seems to be responsible, at least in part, for their low MDP (Sartiani et al., 2007). Cardiomyocytes produced with both methods did not differ in their upstroke velocity (Pekkanen-Mattila et al., 2010a).

Dolnikov and colleagues studied mechanical functions of hESC derived cardiac cells. They found that hESC-CMs have a negative force-frequency relation, whereas mature human myocardium the relationship is positive (Dolnikov et al., 2005). They also found that blocking the ryanodine receptor or the sarcoplasmic-endoplasmic reticulum Ca^{2+} -ATPase did not affect the hESC-CM contraction as it usually does in mature cardiomyocytes. Furthermore, caffeine did not result in increase of intracellular calcium concentration. However, in subsequent studies caffeine-indused release of intracellular Ca^{2+} has been documented both in hESC and hiPSC -derived cardiac cells (Satin et al., 2008, Itzhaki et al., 2011). Both RyR mediated release of intracellular Ca^{2+} stores as well as the reuptake of Ca^{2+} by SERCA into endoplasmic reticulum were reported to occur the same way as in cardiac tissue. These results indicate that Ca^{2+} handling in both hESC and hiPSC-derived cardiac cells are functional and further indicate the potential of these cardiac cells in the future applications in basic research as well as in translational cardiac research.

5.2 Microelectrode array platform

In addition to the more traditional patch clamp (Hamill et al., 1981) studies the MEA platform (Reppel et al., 2004) offers an easy and convenient medium-throughput technique to assess the electrical properties of the differentiated cardiomyocytes (Reppel et al., 2005). Action and field potential curves achieved by patch clamp and by MEA are represented in Figure 3. The MEA platform presents an advantageous additional tool for cardiac safety studies in addition to the more traditional Langendorff heart organ model, conventional patch clamp electrophysiology studies and heterologous expression systems of ion channels, especially the hERG potassium channel (Meyer et al., 2004)..

The MEA system allows examination of multicellular cardiac syncythia, thus enabling electrocardiogram-like mapping of their field potential properties. Cardiac repolarization in hESC-CMs can be therefore investigated with MEAs and it has been demonstrated that drug effects can be investigated using this platform (Reppel et al., 2005). With MEA system, Caspi and co-workers were able to investigate drug effects on hESC-CMs. E-4031 is a compound that blocks I_{Kr} repolarizing current and this effect can be seen as prolongation of the cardiac field potential (FP) cycle in the electrocardiogram. The authors were able to demonstrate a dose-dependent effect where the field potential duration (FPD) prolonged by escalating concentrations of E-4031. Sotalol, a class III antiarrhythmic agent, also increased FPD as did quinidine and procainamide, both class IA antiarrhythmic agents. Cisapride, a gastrointestinal prokinetic drug that was withdrawn for the market due to adverse cardiac side effects, prolonged the FPD as well, as seen on the MEA recordings (Caspi et al., 2009, Liang et al., 2010).

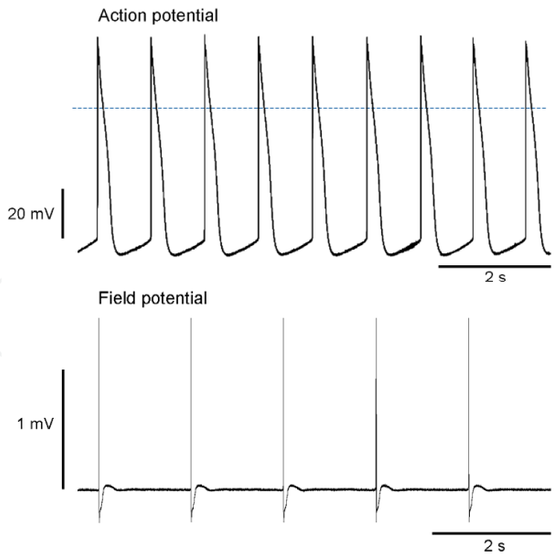


Fig. 3. Action and field potentials recorded from human embryonic stem cell –derived cardiomyocytes. Action potentials can be measured from single cardiomyocytes in current clamp mode using the patch clamp technique. Field potentials can be measured from a multicellular cardiac syncytium with microelectrode arrays.

To assess the practicality of using hESC-CMs in conjunction with the MEA platform in pharmacotoxicological testing, Braam and colleagues investigated the effects of various drugs on the FPD of cardiomyocytes derived from human ES cells (Braam et al., 2010). They tested 12 compounds for their potential in prolonging repolarization (FPD) in hESC-CMs. Despite the relatively lower maturation of hESC-CMs compared to mature cardiomyocytes, they could be used in predicting the clinically observed cardiotoxic effects. Blocking of sodium, calcium and *HERG* potassium channels by lidocaine, nifidipine, and E-4031, respectively, had expected effects on hESC-CM field potential properties. Quinidine and sotalol, both used clinically to prolong repolarization, increased hESC-CM FPD at concentrations near the unbound effective therapeutic plasma concentration (ETCP unbound). They also tested drugs which have been known to prolong the QT interval in patients and noticed that the FPD prolongations too place at concentrations that were not as near the ECTP unbound range. The varying FP shapes in hESC-CM recordings did not affect the results, meaning that FPD changes in these cardiomyocytes can be reliably detected despite the large variety of FP shapes (Braam et al., 2010). The study provided, for the first time, data on the effects of large number of tested compound over a high concentration range on the FP properties of hESC-CM.

6. Applications for pluripotent stem cell-derived cardiomyocytes

6.1 Human model for development of cardiomyocytes and cardiac electrophysiology

The amount of data about electrophysiological changes during human cardiac differentiation is limited. Data is mostly based on animal models, but due to the physiological differences between species this data cannot be always applied directly to humans. Human pluripotent stem cells differentiate into functional cardiomyocytes and these cells also mature in culture (Sartiani et al., 2007). Therefore they provide a good model

to study development of complex network of cardiac ion channels involved in signal transduction and cardiomyocyte contraction.

6.2 Drug screening and safety pharmacology

Many cardiac and also non-cardiac drugs have been withdrawn from the market because of toxic effects on heart and its function. Even though all new chemical entities (NCE) are tested according to requirements during drug development process, unforeseen effects such as syncope, arrhythmia, sudden death, polymorphic ventricular tachycardia (Torsade de pointes [TdP]) are occasionally seen only in clinical trials or when the drug is already on the market (Redfern et al., 2003, Roden, 2004, Lexchin, 2005). Pharmaceutical regulatory authorities have specified and expanded the requirements for safety testing and recommend that tests are done with two mammalian species, one rodent and other nonrodent species. Tests include electocardiographic recordings and also histological studies of the heart (ICH, 2005, ICH, 2005, EMEA, 2008).

With regard to proarrhythmic potential, the QT interval is the cornerstone of the guidelines for the assessment of new chemical compounds (ICH, 2005, ICH, 2005). A number of drugs can potentially prolong the QT interval (Fenichel et al., 2004, Roden, 2004), and it is also a leading cause for use restriction and market withdrawal (Roden, 2004), the International Conference of Harmonization has defined the evaluation of this risk for new chemical entities as standard preclinical process (Bode & Olejniczak, 2002, Cavero & Crumb, 2005).

Delayed rectifier potassium current (I_{Kr}) is responsible in part for the repolarization of the action potential (Vandenberg et al., 2001, Pollard et al., 2008). Inhibition of this hERG channel ($K_{V11.1}$) and the subsequent inhibition of the I_{Kr}, is the predominant basis of drug-induced QT prolongation and TdP (Hancox et al., 2008; Redfern et al., 2003). Currently a number of preclinical models and assays have been employed by pharmaceutical companies (Carlsson, 2006, Pollard et al., 2008). These assays include in vivo QT assays, such as ECG telemetry of conscious dogs (Miyazaki et al., 2005), and in vitro assays, such as repolarization assay, which detects changes in the action potential delay (APD) of cardiac tissues (isolated animal Purkinje fibres, papillary muscles or cardiomyocytes) or the hERG channel assay where hERG current expressed in heterologous cell system (such as CHO or HEK293 cells) or native I_{Kr} is characterized (Finlayson et al., 2004, Martin et al., 2004). However, current methods are not fully adequate (Redfern et al., 2003, Lu et al., 2008). They are costly and the in vivo assays are ethically questionable because of the large number of animals used. Therefore there is a need for an in vitro method based on human cardiac cells that would bring additional value and reliability for testing novel pharmaceutical agents. Cardiomyocytes derived both from hESC and iPS cells have many potential applications in the pharmaceutical industry including target validation, screening and safety pharmacology. These cells would serve as an inexhaustible and reproducible human model system and preliminary reports of the validation of hESC-CM system already exist (Braam et al., 2010, Mandenius et al., 2011).

6.3 Disease modelling with induced pluripotent stem cell –derived cardiomyocytes

The hiPSC technology (Takahashi et al., 2007, Yu et al., 2007) presents a great opportunity to investigate diseases in cell culture that would otherwise be challenging to study. Although the full potential of this method is still to be realized in cardiac research, some preliminary results provide encouragement to investigate this path further.

One condition that is challenging to study in patients in terms of underlying molecular mechanisms is the long QT syndrome (LQTS). This condition can be either genetic or acquired as a side effect of using certain therapeutic drugs. The first LQTS modelling using hiPSCs was reported in 2010 (Moretti et al., 2010). Pluripotent stem cells were reprogrammed from fibroblasts of two family members having LQTS type 1, and cardiac cells derived from these hiPSCs with LQT1 genotype had prolonged action potential. Additionally increased arrhythmogenicity could be demonstrated with isoproterenol. Similar findings have been reported about LQTS type 2 (Itzhaki et al., 2011, Matsa et al., 2011).

Cardiomyocytes have also been derived from hiPSCs from a patient with Timothy syndrome (Yazawa et al., 2011). These patients have mutation in the *CACNA1C* gene that encodes the $Ca_V1.2$ calcium ion channel in humans. The cardiomyocytes having Timothy syndrome genotype exhibited irregular contractions and excessive calcium influx as well as action potential prolongation, irregularities in the electrical activation and abnormal calcium transients in the ventricular-type cardiomyocytes (Yazawa et al., 2011).

Taken together these results provide optimism for modelling different cardiac disease phenotypes in cell culture conditions. This allows for more detailed dissection of the pathological pathways and molecular interactions within and between the cells. An additional benefit is also the fact that these studies can be now carried out in patient-specific cells which provide suitable genomic backgrounds for more optimal comparison between the clinical disease phenotype and results obtained *in vitro*.

7. Challenges in pluripotent stem cell research

While human pluripotent stem cells represent a promising new tool for pharmacological and toxicological testing and hopefully also for regenerative therapies in the future some hurdles remain to be cleared before we can achieve those goals efficiently. With regards to hiPSC a question remains how close these cells are to hESCs in their properties. While they fulfil the criteria required for pluripotent stem cells, some recent studies suggest that they retain some aberrant epigenetic reprogramming compared to ES cells (Lister et al., 2011). Both cell types have similar global methylomes, but the reprogrammed iPS cell seem to retain some memory of the somatic cell DNA methylation patterns in addition to the methylation patterns that are specific for iPSCs (Lister et al., 2011). However, the cardiomyocytes differentiated from hiPSCs and hESCs seem to have very similar global transcriptomes (Gupta et al., 2010).

Another issue with both, hESCs and hiPSCs, are the currently suboptimal differentiation protocols for desired differentiated cell types. Cardiomyocytes are no exception in this case and several protocols for more efficient differentiation have been experimented with. Recently, increased yields of cardiomyocytes have been obtained by stage-specific optimization of the activin/nodal and bone morphogenetic protein (BMP) signalling (Kattman et al., 2011). The directed differentiation protocol of hESC-CMs in a monolayer, with activin A and BMP4 supplementation, represents another step forward in creating more efficient differentiation methods (Laflamme et al., 2007). In hiPS reprogramming omission of c-Myc from the four factor Yamanaka cocktail has been shown to enhance their cardiogenic potential (Martinez-Fernandez et al., 2010).

To achieve better cardiomyocyte differentiation efficiencies with pluripotent stem cells we need to gain more insight into the lineage-specification steps that govern the transformation

of pluripotent stem cells to committed progenitors and finally to mature differentiated cardiomyocytes. For example, $ISL1^+$ cardiac progenitors are able to give rise to cardiomyocytes, smooth muscle, and endothelial cell lineages and these progenitor populations can be expanded in cell culture (Bu et al., 2009). KDR^+ cells derived from human embryonic stem cells have also been shown to give rise to cardiac progenitor cells (Yang et al., 2008).

8. Conclusion

In conclusion, human pluripotent stem cell derived cardiomyocytes have very similar electrophysiological properties as human heart tissue and, thus, they have a great potential in the future to benefit pharmaceutical and toxicological industry. Additionally, with these cells we are closer than ever before to individualized, patient-specific treatments. However, a lot of basic research is still required before we can utilize the full advantage of human pluripotent stem cell –derived cardiac cells.

9. References

Allah, E.A., Tellez, J.O., Yanni, J., Nelson, T., Monfredi, O., Boyett, M.R. & Dobrzynski, H. (2011). Changes in the expression of ion channels, connexins and Ca2+-handling proteins in the sino-atrial node during postnatal development. *Exp Physiol,* Vol. 96, No. 4, (Apr), pp. 426-38

Artman, M. (1992). Sarcolemmal Na(+)-Ca2+ exchange activity and exchanger immunoreactivity in developing rabbit hearts. *Am J Physiol,* Vol. 263, No. 5 Pt 2, (Nov), pp. H1506-13

Artman, M., Ichikawa, H., Avkiran, M. & Coetzee, W.A. (1995). Na+/Ca2+ exchange current density in cardiac myocytes from rabbits and guinea pigs during postnatal development. *Am J Physiol,* Vol. 268, No. 4 Pt 2, (Apr), pp. H1714-22

Asp, J., Steel, D., Jonsson, M., Ameen, C., Dahlenborg, K., Jeppsson, A., Lindahl, A. & Sartipy, P. (2010). Cardiomyocyte clusters derived from human embryonic stem cells share similarities with human heart tissue. *J Mol Cell Biol,* Vol. 2, No. 5, (October 2010), pp. 276-283

Beqqali, A., Kloots, J., Ward-Van Oostwaard, D., Mummery, C. & Passier, R. (2006). Genome-wide transcriptional profiling of human embryonic stem cells differentiating to cardiomyocytes. *Stem Cells,* Vol. No. (May 4, 2006), pp. 2006-0054

Bettiol, E., Sartiani, L., Chicha, L., Krause, K.H., Cerbai, E. & Jaconi, M.E. (2007). Fetal bovine serum enables cardiac differentiation of human embryonic stem cells. *Differentiation,* Vol. 75, No. 8, (Oct), pp. 669-81

Bode, G. & Olejniczak, K. (2002). ICH Topic: The draft ICH S7B step 2: Note for guidance on safety pharmacology studies for human pharmaceuticals. *Fundamental & Clinical Pharmacology,* Vol. 16, No. 2, (April 2002), pp. 105-118

Braam, S.R., Tertoolen, L., Van De Stolpe, A., Meyer, T., Passier, R. & Mummery, C.L. (2010). Prediction of drug-induced cardiotoxicity using human embryonic stem cell-derived cardiomyocytes. *Stem Cell Research,* Vol. 4, No. 2, (March 2010), pp. 107-116

Braam, S.R., Tertoolen, L., Van De Stolpe, A., Meyer, T., Passier, R. & Mummery, C.L. (2010). Prediction of drug-induced cardiotoxicity using human embryonic stem cell-derived cardiomyocytes. *Stem Cell Res,* Vol. 4, No. 2, (Mar), pp. 107-16

Brook, W.H., Connell, S., Cannata, J., Maloney, J.E. & Walker, A.M. (1983). Ultrastructure of the myocardium during development from early fetal life to adult life in sheep. *J Anat,* Vol. 137 (Pt 4), No. (Dec), pp. 729-41

Bu, L., Jiang, X., Martin-Puig, S., Caron, L., Zhu, S., Shao, Y., Roberts, D.J., Huang, P.L., Domian, I.J. & Chien, K.R. (2009). Human ISL1 heart progenitors generate diverse multipotent cardiovascular cell lineages. *Nature,* Vol. 460, No. 7251, (July 2009), pp. 113-117

Buckingham, M., Meilhac, S. & Zaffran, S. (2005). Building the mammalian heart from two sources of myocardial cells. *Nat Rev Genet,* Vol. 6, No. 11, (Nov), pp. 826-35

Burridge, P.W., Anderson, D., Priddle, H., Barbadillo Munoz, M.D., Chamberlain, S., Allegrucci, C., Young, L.E. & Denning, C. (2007). Improved human embryonic stem cell embryoid body homogeneity and cardiomyocyte differentiation from a novel V-96 plate aggregation system highlights interline variability. *Stem Cells,* Vol. 25, No. 4, (Apr), pp. 929-38

Carlsson, L. (2006). In vitro and in vivo models for testing arrhythmogenesis in drugs. *J Intern Med,* Vol. 259, No. 1, (Jan), pp. 70-80

Caspi, O., Itzhaki, I., Kehat, I., Gepstein, A., Arbel, G., Huber, I., Satin, J. & Gepstein, L. (2009). In vitro electrophysiological drug testing using human embryonic stem cell derived cardiomyocytes. *Stem Cells and Development,* Vol. 18, No. 1, (February 2009), pp. 161-172

Cavero, I. & Crumb, W. (2005). ICH S7B draft guideline on the non-clinical strategy for testing delayed cardiac repolarisation risk of drugs: a critical analysis. *Expert Opinion on Drug Safety,* Vol. 4, No. 3, (May 2005), pp. 509-530

Chin, T.K., Christiansen, G.A., Caldwell, J.G. & Thorburn, J. (1997). Contribution of the sodium-calcium exchanger to contractions in immature rabbit ventricular myocytes. *Pediatr Res,* Vol. 41, No. 4 Pt 1, (Apr), pp. 480-5

Dolnikov, K., Shilkrut, M., Zeevi-Levin, N., Danon, A., Gerecht-Nir, S., Itskovitz-Eldor, J. & Binah, O. (2005). Functional properties of human embryonic stem cell-derived cardiomyocytes. *Annals of the New York Academy of Sciences,* Vol. 1047, No. (June 2005), pp. 66-75

Dolnikov, K., Shilkrut, M., Zeevi-Levin, N., Gerecht-Nir, S., Amit, M., Danon, A., Itskovitz-Eldor, J. & Binah, O. (2006). Functional properties of human embryonic stem cell-derived cardiomyocytes: intracellular Ca2+ handling and the role of sarcoplasmic reticulum in the contraction. *Stem Cells,* Vol. 24, No. 2, (Feb), pp. 236-45

Fabiato, A. (1983). Calcium-induced release of calcium from the cardiac sarcoplasmic reticulum. *Am J Physiol,* Vol. 245, No. 1, (Jul), pp. C1-14

Fenichel, R.R., Malik, M., Antzelevitch, C., Sanguinetti, M., Roden, D.M., Priori, S.G., Ruskin, J.N., Lipicky, R.J., Cantilena, L.R. & Independent Academic Task, F. (2004). Drug-Induced Torsades de Pointes and Implications for Drug Development. *Journal of Cardiovascular Electrophysiology,* Vol. 15, No. 4, (April 2004), pp. 475-495

Finlayson, K., Witchel, H.J., Mcculloch, J. & Sharkey, J. (2004). Acquired QT interval prolongation and HERG: implications for drug discovery and development. *Eur J Pharmacol,* Vol. 500, No. 1-3, (Oct 1), pp. 129-42

Freund, C., Ward-Van Oostwaard, D., Monshouwer-Kloots, J., Van Den Brink, S., Van Rooijen, M., Xu, X., Zweigerdt, R., Mummery, C. & Passier, R. (2008). Insulin redirects differentiation from cardiogenic mesoderm and endoderm to neuroectoderm in differentiating human embryonic stem cells. *Stem Cells,* Vol. 26, No. 3, (Mar), pp. 724-33

Fujiwara, M., Yan, P., Otsuji, T.G., Narazaki, G., Uosaki, H., Fukushima, H., Kuwahara, K., Harada, M., Matsuda, H., Matsuoka, S., Okita, K., Takahashi, K., Nakagawa, M., Ikeda, T., Sakata, R., Mummery, C.L., Nakatsuji, N., Yamanaka, S., Nakao, K. & Yamashita, J.K. (2011). Induction and enhancement of cardiac cell differentiation from mouse and human induced pluripotent stem cells with cyclosporin-a. *PLoS ONE,* Vol. 6, No. 2, pp. e16734

Gershome, C., Lin, E., Kashihara, H., Hove-Madsen, L. & Tibbits, G.F. (2011). Colocalization of voltage-gated Na+ channels with the Na+/Ca2+ exchanger in rabbit cardiomyocytes during development. *Am J Physiol Heart Circ Physiol,* Vol. 300, No. 1, (Jan), pp. H300-11

Graichen, R., Xu, X., Braam, S.R., Balakrishnan, T., Norfiza, S., Sieh, S., Soo, S.Y., Tham, S.C., Mummery, C., Colman, A., Zweigerdt, R. & Davidson, B.P. (2008). Enhanced cardiomyogenesis of human embryonic stem cells by a small molecular inhibitor of p38 MAPK. *Differentiation,* Vol. 76, No. 4, (Apr), pp. 357-70

Guo, W., Kamiya, K. & Toyama, J. (1996). Modulated expression of transient outward current in cultured neonatal rat ventricular myocytes: comparison with development in situ. *Cardiovascular Research,* Vol. 32, No. 3, (September 1996), pp. 524-533

Gupta, M.K., Illich, D.J., Gaarz, A., Matzkies, M., Nguemo, F., Pfannkuche, K., Liang, H., Classen, S., Reppel, M., Schultze, J.L., Hescheler, J. & Saric, T. (2010). Global transcriptional profiles of beating clusters derived from human induced pluripotent stem cells and embryonic stem cells are highly similar. *BMC Developmental Biology,* Vol. 10, No. (September 2010), pp. 98

Haddock, P.S., Coetzee, W.A. & Artman, M. (1997). Na+/Ca2+ exchange current and contractions measured under Cl(-)-free conditions in developing rabbit hearts. *Am J Physiol,* Vol. 273, No. 2 Pt 2, (Aug), pp. H837-46

Hamill, O.P., Marty, A., Neher, E., Sakmann, B. & Sigworth, F.J. (1981). Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *European Journal of Physiology,* Vol. 391, No. 2, (August 1981), pp. 85-100

He, J.-Q., Ma, Y., Lee, Y., Thomson, J.A. & Kamp, T.J. (2003). Human Embryonic Stem Cells Develop Into Multiple Types of Cardiac Myocytes: Action Potential Characterization. *Circ Res,* Vol. 93, No. 1, (July 11, 2003), pp. 32-39

Huang, X., Yang, P., Du, Y., Zhang, J. & Ma, A. (2007). Age-related down-regulation of HCN channels in rat sinoatrial node. *Basic Res Cardiol,* Vol. 102, No. 5, (Sep), pp. 429-35

Huynh, T.V., Chen, F., Wetzel, G.T., Friedman, W.F. & Klitzner, T.S. (1992). Developmental changes in membrane Ca2+ and K+ currents in fetal, neonatal, and adult rabbit ventricular myocytes. *Circ Res,* Vol. 70, No. 3, (Mar), pp. 508-15

Itskovitz-Eldor, J., Schuldiner, M., Karsenti, D., Eden, A., Yanuka, O., Amit, M., Soreq, H. & Benvenisty, N. (2000). Differentiation of human embryonic stem cells into embryoid bodies compromising the three embryonic germ layers. *Mol Med,* Vol. 6, No. 2, (Feb), pp. 88-95

Itzhaki, I., Maizels, L., Huber, I., Zwi-Dantsis, L., Caspi, O., Winterstern, A., Feldman, O., Gepstein, A., Arbel, G., Hammerman, H., Boulos, M. & Gepstein, L. (2011). Modelling the long QT syndrome with induced pluripotent stem cells. *Nature,* Vol. 471, No. 7337, (March 2011), pp. 225-259

Kattman, S.J., Witty, A.D., Gagliardi, M., Dubois, N.C., Niapour, M., Hotta, A., Ellis, J. & Keller, G. (2011). Stage-Specific Optimization of Activin/Nodal and BMP Signaling Promotes Cardiac Differentiation of Mouse and Human Pluripotent Stem Cell Lines. *Cell Stem Cell,* Vol. 8, No. 2, (February 2011), pp. 228-240

Kehat, I., Kenyagin-Karsenti, D., Snir, M., Segev, H., Amit, M., Gepstein, A., Livne, E., Binah, O., Itskovitz-Eldor, J. & Gepstein, L. (2001). Human embryonic stem cells can differentiate into myocytes with structural and functional properties of cardiomyocytes. *The Journal of Clinical Investigation,* Vol. 108, No. 3, (August 2001), pp. 407-414

Kehat, I., Kenyagin-Karsenti, D., Snir, M., Segev, H., Amit, M., Gepstein, A., Livne, E., Binah, O., Itskovitz-Eldor, J. & Gepstein, L. (2001). Human embryonic stem cells can differentiate into myocytes with structural and functional properties of cardiomyocytes. *J Clin Invest,* Vol. 108, No. 3, (Aug), pp. 407-14

Klitzner, T.S., Chen, F.H., Raven, R.R., Wetzel, G.T. & Friedman, W.F. (1991). Calcium current and tension generation in immature mammalian myocardium: effects of diltiazem. *J Mol Cell Cardiol,* Vol. 23, No. 7, (Jul), pp. 807-15

Klitzner, T.S. & Friedman, W.F. (1989). A diminished role for the sarcoplasmic reticulum in newborn myocardial contraction: effects of ryanodine. *Pediatr Res,* Vol. 26, No. 2, (Aug), pp. 98-101

Laflamme, M.A., Chen, K.Y., Naumova, A.V., Muskheli, V., Fugate, J.A., Dupras, S.K., Reinecke, H., Xu, C., Hassanipour, M., Police, S., O'sullivan, C., Collins, L., Chen, Y., Minami, E., Gill, E.A., Ueno, S., Yuan, C., Gold, J. & Murry, C.E. (2007). Cardiomyocytes derived from human embryonic stem cells in pro-survival factors enhance function of infarcted rat hearts. *Nature Biotechnology,* Vol. 25, No. 9, (September 2007), pp. 1015-1024

Laflamme, M.A., Chen, K.Y., Naumova, A.V., Muskheli, V., Fugate, J.A., Dupras, S.K., Reinecke, H., Xu, C., Hassanipour, M., Police, S., O'sullivan, C., Collins, L., Chen, Y., Minami, E., Gill, E.A., Ueno, S., Yuan, C., Gold, J. & Murry, C.E. (2007). Cardiomyocytes derived from human embryonic stem cells in pro-survival factors enhance function of infarcted rat hearts. *Nat Biotechnol,* Vol. 25, No. 9, (Sep), pp. 1015-24

Lexchin, J. (2005). Drug withdrawals from the Canadian market for safety reasons, 1963-2004. *Cmaj,* Vol. 172, No. 6, (Mar 15), pp. 765-7

Liang, H., Matzkies, M., Schunkert, H., Tang, M., Bonnemeier, H., Hescheler, J. & Reppel, M. (2010). Human and murine embryonic stem cell-derived cardiomyocytes serve together as a valuable model for drug safety screening. *Cell Physiol Biochem,* Vol. 25, No. 4-5, (March 2010), pp. 459-466

Lister, R., Pelizzola, M., Kida, Y.S., Hawkins, R.D., Nery, J.R., Hon, G., Antosiewicz-Bourget, J., O'malley, R., Castanon, R., Klugman, S., Downes, M., Yu, R., Stewart, R., Ren, B., Thomson, J.A., Evans, R.M. & Ecker, J.R. (2011). Hotspots of aberrant epigenomic reprogramming in human induced pluripotent stem cells. *Nature,* Vol. 471, No. 7336, (March 2011), pp. 68-73

Lough, J. & Sugi, Y. (2000). Endoderm and heart development. *Dev Dyn,* Vol. 217, No. 4, (Apr), pp. 327-42

Lu, H.R., Vlaminckx, E., Hermans, A.N., Rohrbacher, J., Van Ammel, K., Towart, R., Pugsley, M. & Gallacher, D.J. (2008). Predicting drug-induced changes in QT interval and arrhythmias: QT-shortening drugs point to gaps in the ICHS7B Guidelines. *Br J Pharmacol,* Vol. 154, No. 7, (Aug), pp. 1427-38

Mandenius, C.F., Steel, D., Noor, F., Meyer, T., Heinzle, E., Asp, J., Arain, S., Kraushaar, U., Bremer, S., Class, R. & Sartipy, P. (2011). Cardiotoxicity testing using pluripotent stem cell-derived human cardiomyocytes and state-of-the-art bioanalytics: a review. *J Appl Toxicol,* Vol. 31, No. 3, (Apr), pp. 191-205

Martin, R.L., Mcdermott, J.S., Salmen, H.J., Palmatier, J., Cox, B.F. & Gintant, G.A. (2004). The utility of hERG and repolarization assays in evaluating delayed cardiac repolarization: influence of multi-channel block. *J Cardiovasc Pharmacol,* Vol. 43, No. 3, (Mar), pp. 369-79

Martinez-Fernandez, A., Nelson, T.J., Ikeda, Y. & Terzic, A. (2010). c-MYC independent nuclear reprogramming favors cardiogenic potential of induced pluripotent stem cells. *Journal of Cardiovascular Translational Research,* Vol. 3, No. 1, (February 2010), pp. 13-23

Matsa, E., Rajamohan, D., Dick, E., Young, L., Mellor, I., Staniforth, A. & Denning, C. (2011). Drug evaluation in cardiomyocytes derived from human induced pluripotent stem cells carrying a long QT syndrome type 2 mutation. *Eur Heart J,* Vol. 32, No. 8, (April 2011), pp. 952-962

Meyer, T., Boven, K.H., Gunther, E. & Fejtl, M. (2004). Micro-electrode arrays in cardiac safety pharmacology: a novel tool to study QT interval prolongation. *Drug Safety,* Vol. 27, No. 11, (n.d.), pp. 763-72

Miyazaki, H., Watanabe, H., Kitayama, T., Nishida, M., Nishi, Y., Sekiya, K., Suganami, H. & Yamamoto, K. (2005). QT PRODACT: sensitivity and specificity of the canine telemetry assay for detecting drug-induced QT interval prolongation. *J Pharmacol Sci,* Vol. 99, No. 5, pp. 523-9

Moretti, A., Bellin, M., Welling, A., Jung, C.B., Lam, J.T., Bott-Flugel, L., Dorn, T., Goedel, A., Hohnke, C., Hofmann, F., Seyfarth, M., Sinnecker, D., Schomig, A. & Laugwitz, K.L. (2010). Patient-specific induced pluripotent stem-cell models for long-QT syndrome. *N Engl J Med,* Vol. 363, No. 15, (October 2010), pp. 1397-1409

Mummery, C., Ward-Van Oostwaard, D., Doevendans, P., Spijker, R., Van Den Brink, S., Hassink, R., Van Der Heyden, M., Opthof, T., Pera, M., De La Riviere, A.B., Passier,

R. & Tertoolen, L. (2003). Differentiation of human embryonic stem cells to cardiomyocytes: role of coculture with visceral endoderm-like cells. *Circulation,* Vol. 107, No. 21, (Jun 3), pp. 2733-40

Nakanishi, T., Okuda, H., Kamata, K., Abe, K., Sekiguchi, M. & Takao, A. (1987). Development of myocardial contractile system in the fetal rabbit. *Pediatr Res,* Vol. 22, No. 2, (Aug), pp. 201-7

Nassar, R., Reedy, M.C. & Anderson, P.A. (1987). Developmental changes in the ultrastructure and sarcomere shortening of the isolated rabbit ventricular myocyte. *Circ Res,* Vol. 61, No. 3, (Sep), pp. 465-83

Nerbonne, J.M. & Kass, R.S. (2005). Molecular physiology of cardiac repolarization. *Physiol Rev,* Vol. 85, No. 4, (October 2005), pp. 1205-1253

Passier, R., Oostwaard, D.W., Snapper, J., Kloots, J., Hassink, R.J., Kuijk, E., Roelen, B., De La Riviere, A.B. & Mummery, C. (2005). Increased cardiomyocyte differentiation from human embryonic stem cells in serum-free cultures. *Stem Cells,* Vol. 23, No. 6, (Jun-Jul), pp. 772-80

Pekkanen-Mattila, M., Chapman, H., Kerkela, E., Suuronen, R., Skottman, H., Koivisto, A.P. & Aalto-Setala, K. (2010). Human embryonic stem cell-derived cardiomyocytes: demonstration of a portion of cardiac cells with fairly mature electrical phenotype. *Experimental Biology and Medicine,* Vol. 235, No. 4, (April 2010), pp. 522-530

Pekkanen-Mattila, M., Kerkela, E., Tanskanen, J.M., Pietila, M., Pelto-Huikko, M., Hyttinen, J., Skottman, H., Suuronen, R. & Aalto-Setala, K. (2009). Substantial variation in the cardiac differentiation of human embryonic stem cell lines derived and propagated under the same conditions--a comparison of multiple cell lines. *Ann Med,* Vol. 41, No. 5, pp. 360-70

Pekkanen-Mattila, M., Kerkelä, E., Tanskanen, J.M.A., Pietilä, M., Pelto-Huikko, M., Hyttinen, J., Skottman, H., Suuronen, R. & Aalto-Setälä, K. (2009). Substantial variation in the cardiac differentiation of human embryonic stem cell lines derived and propagated under the same conditions: a comparison of multiple cell lines. *Annals of Medicine,* Vol. 41, No. 5, (n.d.), pp. 360-370

Pekkanen-Mattila, M., Pelto-Huikko, M., Kujala, V., Suuronen, R., Skottman, H., Aalto-Setala, K. & Kerkela, E. (2010). Spatial and temporal expression pattern of germ layer markers during human embryonic stem cell differentiation in embryoid bodies. *Histochem Cell Biol,* Vol. 133, No. 5, (May), pp. 595-606

Pera, M.F. & Trounson, A.O. (2004). Human embryonic stem cells: prospects for development. *Development,* Vol. 131, No. 22, (Nov), pp. 5515-25

Pollard, C.E., Abi Gerges, N., Bridgland-Taylor, M.H., Easter, A., Hammond, T.G. & Valentin, J.P. (2010). An introduction to QT interval prolongation and non-clinical approaches to assessing and reducing risk. *British Journal of Pharmacology,* Vol. 159, No. 1, (January 2010), pp. 12-21

Pollard, C.E., Valentin, J.P. & Hammond, T.G. (2008). Strategies to reduce the risk of drug-induced QT interval prolongation: a pharmaceutical company perspective. *Br J Pharmacol,* Vol. 154, No. 7, (Aug), pp. 1538-43

Redfern, W.S., Carlsson, L., Davis, A.S., Lynch, W.G., Mackenzie, I., Palethorpe, S., Siegl, P.K., Strang, I., Sullivan, A.T., Wallis, R., Camm, A.J. & Hammond, T.G. (2003).

Relationships between preclinical cardiac electrophysiology, clinical QT interval prolongation and torsade de pointes for a broad range of drugs: evidence for a provisional safety margin in drug development. *Cardiovasc Res,* Vol. 58, No. 1, (Apr 1), pp. 32-45

Reppel, M., Pillekamp, F., Brockmeier, K., Matzkies, M., Bekcioglu, A., Lipke, T., Nguemo, F., Bonnemeier, H. & Hescheler, J. (2005). The electrocardiogram of human embryonic stem cell-derived cardiomyocytes. *Journal of Electrocardiology,* Vol. 38, No. 4, Supplement 1, (October 2005), pp. 166-170

Reppel, M., Pillekamp, F., Lu, Z.J., Halbach, M., Brockmeier, K., Fleischmann, B.K. & Hescheler, J. (2004). Microelectrode arrays: A new tool to measure embryonic heart activity. *Journal of Electrocardiology,* Vol. 37, No. Supplement 1, (October 2004), pp. 104-109

Roden, D.M. (2004). Drug-Induced Prolongation of the QT Interval. *New England Journal of Medicine,* Vol. 350, No. 10, (March 2004), pp. 1013-1022

Roden, D.M. (2004). Drug-induced prolongation of the QT interval. *N Engl J Med,* Vol. 350, No. 10, (Mar 4), pp. 1013-22

Sakmann, B. & Neher, E. (1984). Patch clamp techniques for studying ionic channels in excitable membranes. *Annual Review of Physiology,* Vol. 46, No. (n.d.), pp. 455-72

Sartiani, L., Bettiol, E., Stillitano, F., Mugelli, A., Cerbai, E. & Jaconi, M.E. (2007). Developmental changes in cardiomyocytes differentiated from human embryonic stem cells: a molecular and electrophysiological approach. *Stem Cells,* Vol. 25, No. 5, (May 2007), pp. 1136-1144

Sartiani, L., Bettiol, E., Stillitano, F., Mugelli, A., Cerbai, E. & Jaconi, M.E. (2007). Developmental changes in cardiomyocytes differentiated from human embryonic stem cells: a molecular and electrophysiological approach. *Stem Cells,* Vol. 25, No. 5, (May), pp. 1136-44

Satin, J., Itzhaki, I., Rapoport, S., Schroder, E.A., Izu, L., Arbel, G., Beyar, R., Balke, C.W., Schiller, J. & Gepstein, L. (2008). Calcium handling in human embryonic stem cell-derived cardiomyocytes. *Stem Cells,* Vol. 26, No. 8, (August 2008), pp. 1961-1972

Satin, J., Itzhaki, I., Rapoport, S., Schroder, E.A., Izu, L., Arbel, G., Beyar, R., Balke, C.W., Schiller, J. & Gepstein, L. (2008). Calcium handling in human embryonic stem cell-derived cardiomyocytes. *Stem Cells,* Vol. 26, No. 8, (Aug), pp. 1961-72

Shimoni, Y., Fiset, C., Clark, R.B., Dixon, J.E., Mckinnon, D. & Giles, W.R. (1997). Thyroid hormone regulates postnatal expression of transient K+ channel isoforms in rat ventricle. *Journal of Physiology,* Vol. 500, No. 1, (April 1997), pp. 65-73

Silva, J. & Rudy, Y. (2003). Mechanism of Pacemaking in IK1-Downregulated Myocytes. *Circulation Research,* Vol. 92, No. 3, (February 2003), pp. 261-263

Snir, M., Kehat, I., Gepstein, A., Coleman, R., Itskovitz-Eldor, J., Livne, E. & Gepstein, L. (2003). Assessment of the ultrastructural and proliferative properties of human embryonic stem cell-derived cardiomyocytes. *Am J Physiol Heart Circ Physiol,* Vol. 285, No. 6, (Dec), pp. H2355-63

Sperelakis, N. & Shigenobu, K. (1972). Changes in membrane properties of chick embryonic hearts during development. *J Gen Physiol,* Vol. 60, No. 4, (Oct), pp. 430-53

Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K. & Yamanaka, S. (2007). Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors. *Cell,* Vol. 131, No. 5, (November 2007), pp. 861-872

Thomson, J.A., Itskovitz-Eldor, J., Shapiro, S.S., Waknitz, M.A., Swiergiel, J.J., Marshall, V.S. & Jones, J.M. (1998). Embryonic stem cell lines derived from human blastocysts. *Science,* Vol. 282, No. 5391, (November 1998), pp. 1145-1147

Toda, N. (1980). Age-related changes in the transmembrane potential of isolated rabbit sino-atrial nodes and atria. *Cardiovasc Res,* Vol. 14, No. 1, (Jan), pp. 58-63

Tran, T.H., Wang, X., Browne, C., Zhang, Y., Schinke, M., Izumo, S. & Burcin, M. (2009). Wnt3a-induced mesoderm formation and cardiomyogenesis in human embryonic stem cells. *Stem Cells,* Vol. 27, No. 8, (Aug), pp. 1869-78

Vandenberg, J.I., Walker, B.D. & Campbell, T.J. (2001). HERG K+ channels: friend and foe. *Trends Pharmacol Sci,* Vol. 22, No. 5, (May), pp. 240-6

Wetzel, G.T., Chen, F., Friedman, W.F. & Klitzner, T.S. (1991). Calcium current measurements in acutely isolated neonatal cardiac myocytes. *Pediatr Res,* Vol. 30, No. 1, (Jul), pp. 83-8

Wetzel, G.T., Chen, F. & Klitzner, T.S. (1991). L- and T-type calcium channels in acutely isolated neonatal and adult cardiac myocytes. *Pediatr Res,* Vol. 30, No. 1, (Jul), pp. 89-94

Xu, X.Q., Graichen, R., Soo, S.Y., Balakrishnan, T., Rahmat, S.N., Sieh, S., Tham, S.C., Freund, C., Moore, J., Mummery, C., Colman, A., Zweigerdt, R. & Davidson, B.P. (2008). Chemically defined medium supporting cardiomyocyte differentiation of human embryonic stem cells. *Differentiation,* Vol. 76, No. 9, (Nov), pp. 958-70

Yang, L., Soonpaa, M.H., Adler, E.D., Roepke, T.K., Kattman, S.J., Kennedy, M., Henckaerts, E., Bonham, K., Abbott, G.W., Linden, R.M., Field, L.J. & Keller, G.M. (2008). Human cardiovascular progenitor cells develop from a KDR+ embryonic-stem-cell-derived population. *Nature,* Vol. 453, No. 7194, (May 22), pp. 524-8

Yang, L., Soonpaa, M.H., Adler, E.D., Roepke, T.K., Kattman, S.J., Kennedy, M., Henckaerts, E., Bonham, K., Abbott, G.W., Linden, R.M., Field, L.J. & Keller, G.M. (2008). Human cardiovascular progenitor cells develop from a KDR+ embryonic-stem-cell-derived population. *Nature,* Vol. 453, No. 7194, (May 2008), pp. 524-528

Yazawa, M., Hsueh, B., Jia, X., Pasca, A.M., Bernstein, J.A., Hallmayer, J. & Dolmetsch, R.E. (2011). Using induced pluripotent stem cells to investigate cardiac phenotypes in Timothy syndrome. *Nature,* Vol. 471, No. 7337, (March 2011), pp. 230-234

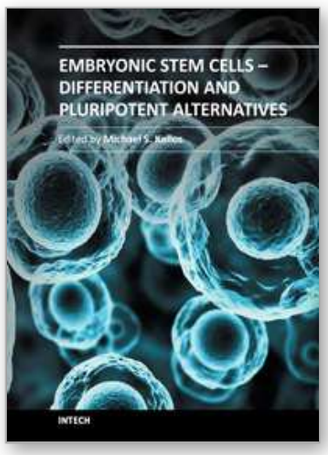
Yu, J., Vodyanik, M.A., Smuga-Otto, K., Antosiewicz-Bourget, J., Frane, J.L., Tian, S., Nie, J., Jonsdottir, G.A., Ruotti, V., Stewart, R., Slukvin, I.I. & Thomson, J.A. (2007). Induced Pluripotent Stem Cell Lines Derived from Human Somatic Cells. *Science,* Vol. 318, No. 5858, (December 2007), pp. 1917-1920

Zhang, J., Wilson, G.F., Soerens, A.G., Koonce, C.H., Yu, J., Palecek, S.P., Thomson, J.A. & Kamp, T.J. (2009). Functional cardiomyocytes derived from human induced pluripotent stem cells. *Circ Res,* Vol. 104, No. 4, (Feb 27), pp. e30-41

Zhang, J., Wilson, G.F., Soerens, A.G., Koonce, C.H., Yu, J., Palecek, S.P., Thomson, J.A. & Kamp, T.J. (2009). Functional cardiomyocytes derived from human induced

pluripotent stem cells. *Circulation Research,* Vol. 104, No. 4, (February 2009), pp. e30-e41

Zwi, L., Caspi, O., Arbel, G., Huber, I., Gepstein, A., Park, I.H. & Gepstein, L. (2009). Cardiomyocyte differentiation of human induced pluripotent stem cells. *Circulation,* Vol. 120, No. 15, (October 2009), pp. 1513-1523



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The ultimate clinical implementation of embryonic stem cells will require methods and protocols to turn these unspecialized cells into the fully functioning cell types found in a wide variety of tissues and organs. In order to achieve this, it is necessary to clearly understand the signals and cues that direct embryonic stem cell differentiation. This book provides a snapshot of current research on the differentiation of embryonic stem cells to a wide variety of cell types, including neural, cardiac, endothelial, osteogenic, and hepatic cells. In addition, induced pluripotent stem cells and other pluripotent stem cell sources are described. The book will serve as a valuable resource for engineers, scientists, and clinicians as well as students in a wide range of disciplines.

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InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
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www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

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