Published in final edited form as:

Adv Drug Deliv Rev. 2016 January 15; 96: 110–134. doi:10.1016/j.addr.2015.04.019.

Towards adult-like human engineered cardiac tissue:

Maturing human pluripotent stem cell-derived cardiomyocytes in human engineered cardiac tissues

Nicole T. Feric¹ and Milica Radisic^{1,2,3}

¹Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, ON, Canada

²Department of Chemical Engineering and Applied Chemistry, University of Toronto, Toronto, ON, Canada

³Toronto General Research Institute, University Health Network, University of Toronto, Toronto, ON, Canada

Abstract

Engineering functional human cardiac tissue that mimics the native adult morphological and functional phenotype has been a long held objective. In the last 5 years, the field of cardiac tissue engineering has transitioned from cardiac tissues derived from various animal species to the production of the first generation of human engineered cardiac tissues (hECTs), due to recent advances in human stem cell biology. Despite this progress, the hECTs generated to date remain immature relative to the native adult myocardium. In this review, we focus on the maturation challenge in the context of hECTs, the present state of the art, and future perspectives in terms of regenerative medicine, drug discovery, preclinical safety testing and pathophysiological studies.

Graphical abstract

Correspondence to: Milica Radisic, Ph.D., P.Eng., Professor and Canada Research Chair (Tier 2), Institute of Biomaterials and Biomedical Engineering, Department of Chemical Engineering and Applied Chemistry, University of Toronto, 164 College St, Rm. 407, Toronto, Ontario, M5S 3G9, m.radisic@utoronto.ca, Phone: 416-946-5295, Fax: 416-978-4317.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

maturation; human engineered cardiac tissue; pluripotent stem cells-derived cardiomyocytes; drug toxicity; target validation; regenerative medicine

1. Introduction

Cardiovascular disease (CVD) is currently the leading cause of death in the world [1], with ischaemic heart disease accounting for the majority of deaths over the past 10 yrs [2]. Unfortunately, that number is projected to continue to rise in the years to come [3]. In part, this is because damage to the myocardium due to ischaemic heart disease (myocardial infarction) does not currently have a curative treatment aside from left ventricular assist devices (LVADs) and/or organ transplantation, which are an option for a limited number of severe cases; and because over 50% of heart disease patients are nonresponsive to the currently available drug therapies [4]. As a consequence, there is a large number of individuals experiencing the debilitating and ultimately fatal effects of heart failure. Hence, there is a need for novel and individualized therapeutic strategies, e.g. disease-specific or patient-specific drugs, and cardiac tissues for regenerative medicine [5].

Cardiotoxicity is one of the principle forms of drug toxicity. It accounts for the majority of drug recalls and regulatory approval delays. Recently, numerous non-cardiac drugs, e.g. terfenadine, have had to be withdrawn from major markets because of concerns of cardiotoxicity. Still others have been withdrawn prior to marketing, while another subset have required label changes that significantly restricted their use [6]. This phenomenon is attributable to the fact that the current drug testing strategies have inherent limitations. They rely upon animal testing, however there are fundamental differences in the electrophysiological properties of animal and human CMs that limit the relevance of pre-clinical animal studies [7]; and human clinical trials have limited applicability due to the necessarily small sample size and the frequent lack of genetic and phenotypic variability. Thus, there is a need for improved preclinical drug screening assays, specifically in discerning cardiotoxic effects and evaluating the efficacy of drug candidates.

Twenty years ago, the successful engineering of a cardiac tissue from embryonic chicken cardiomyocytes (CMs) [8] gave birth to 3-dimensional (3D) cardiac tissue engineering. This field developed with the objectives of producing *in vitro* surrogates of cardiac tissue for *in vivo* repair and preclinical drug development, and of advancing *in vitro* models of heart function and disease [9]. To date, 3D engineered cardiac tissues (ECTs) have not entered the clinical arena nor have they found wide application in target validation and preclinical drug screening. However, a stacked (non-cardiac) cell sheet patch has been used in patient treatment [10] and there are 3D assays ready for application in automated drug testing. Moreover, recent cardiac cell therapy studies have had only limited success in restoring myocardial function, suggesting the need for alternative cell delivery methods or else a novel regenerative approach. In addition to challenges associated with cell retention, injecting dissociated cells into the injured myocardium can induce anoikis [11] and markedly reduce CM function [12]. Conversely, delivery of an intact ECT—cardiac cells seeded in a 3D biodegradable scaffold—to the damaged myocardium could restore

ventricular function if the cells become mature CMs that can beat synchronously with the heart. Proof-of-concept experiments using rodent ECTs have demonstrated the utility of ECTs in elucidating basic principles of myocardial biology and in the development of organ-specific *in vitro* models for drug candidate evaluation, as well as the potential of ECTs as a regenerative therapy to partially or fully restore cardiac function [13–19].

Recent progress in stem cell biology has enabled the widespread availability of human pluripotent stem cell-derived CMs (hPSC-CMs). Human PSC-CMs are generally differentiated by timed application of cardiogenic growth factors or small molecules with cells cultivated as either embryoid bodies (EBs) [20, 21] or in monolayers [22–25], and yield primarily CMs of the ventricular subtype. To produce ECTs the cells are typically dissociated between day 12 and 21 of differentiation and seeded into hydrogels or biomaterials followed by the application of a specific physical stimuli.

However, hPSC-CMs have a markedly immature phenotype at the end of the hPSC differentiation stage. In terms of morphology, gap junction expression, contractile apparatus, spontaneous automaticity, electrophysiology and calcium handling properties, hPSC-CMs are a better approximation of fetal CMs than adult [26]. The utility of ECTs as a model for myocardial development or surrogate for adult tissue in drug development, the objectives as outlined above, depends on its close resemblance to bona fide heart muscle, i.e. its ability to reproduce the morphological and functional properties of mature adult cardiac tissue [27–29]. Signs of hPSC-CMs maturation have been demonstrated through various means, including electrical stimulation [13, 30, 31], mechanical stretching [13, 32–34], construct stiffness and topology [30, 35], and chemical manipulation [29, 34]. The lack of robust methods to promote the functional maturation of hPSC-CMs is currently one of the critical obstacles in the clinical application of ECTs and their use in preclinical drug development.

We review here the challenges in the field of human cardiac tissue engineering, the present state of the art of hECTs, and future perspectives. We will focus on the definition of CM maturity and the properties used to assess CM maturity as it applies to hECTs, and describe the level of maturation achieved in hECTs to date.

2. Cardiac Cell Maturity

Human PSC-CMs resemble human fetal CMs based on gene expression [36], electrophysiology [37] and morphology [38]. Relative to adult CMs, hPSC-CMs are small in size, have reduced electrical excitability [39, 40], impaired excitation-contraction coupling [41, 42] and incomplete adrenergic sensitivity [29, 43]. In the stem cell field, there is no current consensus as to what constitutes a mature adult CM or which markers can be used to accurately and specifically track the maturity of hPSC-CMs. This is primarily because isolated primary CMs can re-express embryonic/fetal isoforms when cultured *in vitro*, and various structural or physiological markers —e.g. Ca^{2+} handling, cell morphology/striation pattern, and beating—undergo developmental reversion under conditions of pathological hypertrophy or disease [44]. The majority of hPSC cardiogenic differentiation protocols generate primarily CMs with a ventricular phenotype. Therefore in the following section, we will outline the different parameters used to assess the maturity of ventricular CMs in human

ECTs (hECTs). For the sake of simplicity, only the non-failing human heart will be considered for comparison.

2.1. Morphology, proliferation and structural properties

Shape: Adult CMs have an elongated anisotropic rod-like shape with a length-to-width (aspect) ratio in the range of 7:1 to 9.5:1 [45] (Figure 1C). Conversely, hPSC-CMs are heterogeneous in shape—polygonal or spherical, smaller in size and have lower aspect ratios [43, 46, 47] (Figure 1A). Upon dissociation, this distinction in shape becomes more apparent because the rigid, highly-organized internal architecture of adult CMs enables them to maintain their rod shape, while hPSC-CMs lack this degree of internal organization and round up. CM shape is not only important as an indicator of internal organization but has functional consequences for excitation-contraction coupling, specifically conduction velocity.

Proliferative Capacity: During development, the proliferative capacity of CMs decreases as CMs withdraw from the cell cycle [48, 49]. In humans, CMs cease to proliferate after the first few postnatal months [50], although a limited capacity to proliferate (~0.5% per year) has been demonstrated in a recent study [51]. A non-proliferative phenotype is considered an indication of terminal differentiation [51].

Binucleation: As CMs transition to a non-proliferative state, many CMs become binucleated. In humans, the proportion of binucleated cells in the heart is ~25% at birth and remains consistent through to adulthood [51] (Figure 1C).

Cell Surface Area: In culture, human adult ventricular CMs have been reported to have a surface area of 10,212–14,418 μm^2 [52] and human fetal CMs of 1171–1261 μm^2 [53]. The reported surface area of hPSC-CMs is within approximately the same range as fetal CMs but 9- to 13-fold smaller than adult CMs, under standard culture conditions (995–1294 μm^2 , [53]). Cell size is functionally significant because a variety of properties—e.g. impulse propagation, action potential depolarization velocity, total contractile force and membrane capacitance—are directly related to cell surface area [54]. An increase in CM size is part of the hypertrophic response of CMs, along with increased protein synthesis and enhanced sarcomeric organization. Two different hypertrophic phenotypes have been characterized: concentric and eccentric [55]. In concentric hypertrophy, pressure overload induces the addition of sarcomeres in parallel, i.e. lateral CM growth [55]. In eccentric hypertrophy, volume overload induces the addition of sarcomeres in series, i.e. longitudinal CM growth [55]. Hypertrophy enables CMs to increase their work output, which improves cardiac pump function. However, hypertrophy is also associated with various disease states. In pathological hypertrophy, fetal genes—e.g. atrial natriuretic peptide (ANP), β -myosin heavy chain (β -MHC), and skeletal α -actin (SKA)—are re-expressed, whereas in physiological hypertrophy they are not [56].

Polyploidy: Coincident with hypertrophy, the DNA content of most nuclei is duplicated (2N to 4N). In humans, the DNA content of CMs is typically diploid at birth but the majority of adult CM nuclei are tetraploid [51].

Cell Alignment & Orientation: In native cardiac tissue, CMs are oriented to form highly aligned myofibers, whereas human embryonic stem cell-derived cardiomyocytes (hESC-CMs) have poor cellular alignment and orientation under standard culture, e.g. EB or monolayer culture [43] (Figure 1). CM alignment has been demonstrated to be important for electromechanical coupling and the generation of contractile force [57, 58].

Sarcomeric Size, Alignment & Organization: Sarcomeric length, alignment, organization and abundance all increase with CM maturity. The average length of a sarcomere in a relaxed adult CM is 2.0–2.2 μ m [59] and 1.8 μ m in a fetal CM [53]. Sarcomeres in hPSC-CMs are 25–30% shorter than in adult CMs and 6–11% smaller than in fetal CMs (1.6–1.7 μ m [60]) (Figure 2). Long-term culture (80–120 days) increased the length of the hPSC-CM sarcomeres to that of fetal CMs [60]. In adult CMs, myofibrils have near perfect alignment of distinct, well-developed and abundant sarcomeres that are distributed throughout the adult tissue. Histologically, this is evident as sharp, highly aligned and organized Z-disc structures of uniform width (Figure 1B & Figure 2A–B). In hPSC-CMs, sarcomeres are often seen as underdeveloped, unaligned Z-discs of variable width [46, 47] (Figure 2C–E). The number of sarcomeres is also significantly reduced and there is an uneven distribution throughout the cell, i.e. more sarcomeres concentrated in the perinuclear region than the cell periphery [23, 25, 37, 61, 62]. Compared with fetal CMs, hPSC-CMs have less defined sarcomeric structures and reduced organization [37, 53]. Sarcomeric organization has been demonstrated to be directly related to action potential duration (APD), Ca^{2+} transient kinetics and contractile stress [53, 63].

It has been particularly challenging to obtain M-lines in hPSC-CMs. An M-line is a histological structure—a thin dark line in the centre of the H-band (or larger A-band) of the sarcomere—denoting where the thick myosin filaments of the A-band are cross-linked (Figure 2A–B). Evidence of M-lines is considered to be an indication of sarcomeric structural maturation. For hPSC-CMs, 360 days of culture were required for M-lines to become visible [64].

Transverse Tubules: T-tubules are invaginations in the CM plasma membrane (sarcolemma), along the boundary between adjacent sarcomeres responsible for transmitting the action potential from the sarcolemma to the sarcoplasmic reticulum (SR). In adult ventricular CMs, transverse tubules (T-tubules) have a uniform spacing of $\sim 2\mu$ m [47], whereas hESC-CMs have few to no T-tubules [65, 66] (Figure 2). The frequency and pattern of T-tubules in hESC-CMs approaches the early T-tubule development seen in fetal or neonatal CMs [67, 68]. T-tubules are critical to effective excitation-contraction coupling and their absence has been associated with non-uniform Ca^{2+} wavefronts in hESC-CMs, i.e. a faster, larger increase in cytosolic Ca^{2+} at the cell periphery than the centre [47, 69]. This Ca^{2+} transient pattern has also been reported for fetal and neonatal CMs lacking T-tubules [69].

Connexin-43 & N-Cadherin: Adult CMs are interconnected at their longitudinal edge by intercalated discs composed of several interacting protein structures—e.g. gap junctions, adherens junctions and desmosomes—that enable electro-mechanical coupling [70]. Connexin-43 is the predominant cardiac gap junction protein. Gap junctions are clusters of

closely-packed transmembrane intercellular channels that enable rapid propagation of the action potential by permitting the movement of low molecular weight materials between cells [70] (Figure 1C). N-cadherin is an important adherens junction protein. Adherens junctions enable the transmission of contractile force from one cell to another [71]. In adult cardiac tissue, action potential propagation is anisotropic due to the high concentration of gap junctions in the intercalated discs and low concentration at the lateral cell CM borders [72]. In fetal cardiac tissue, and similarly in hPSC-CMs, connexin-43 and N-cadherin are distributed throughout the CM membrane and often in gap junctions at the lateral CM borders [16, 26, 73] (Figure 1F). The phenotypically adult membrane distribution of connexin-43 is observed in human cardiac tissue after 6 years of development [74]. The redistribution of connexin-43 and N-cadherin to the intercalated discs has been associated with increased conduction velocity [75].

2.2. Metabolism

During the early phase of embryogenesis, the uterine environment in which the fetal heart develops is oxygen-poor; as such, carbohydrates are the primary source of energy [76]. With maturation of the embryonic circulation the uterine environment becomes increasingly oxygen-rich, and the more energy-efficient process of oxidative phosphorylation begins to supply additional energy needed by the growing heart [77]. In the oxygen-rich postnatal environment there is a shift to long-chain fatty acids as the primary energy source [78]. The number of mitochondria increase with the growing energy demand of the fetus, and the morphology of the mitochondria changes with the developmental stage [77]. In early development mitochondria are round, dilated, lack cristae, are localized to the perinucleus and have minimal interaction with the immature contractile apparatus. Coincident with the transition to aerobic respiration, mitochondria begin to develop cristae and elongate, and begin to form a network in proximity to the contractile machinery. In adult CMs, mitochondria are larger with well-developed cristae and are regularly aligned with the SR and contractile machinery.

In the adult heart under normal conditions, fat provides 60% of the basal energy and anaerobic metabolism accounts for <1% [79]. In adult CMs, glycogen occupies ~2% of the CM volume [78] and mitochondria account for ~20–40% of the cell volume; and mitochondria are tightly packed along myofibrils or beneath the sarcolemma and have distinct, regularly distributed lamellar cristae [80]. In fetal CMs and hPSC-CMs, glycogen occupies >30% of the cell volume [78] and mitochondria account for a small fraction of cell volume; mitochondria are found throughout the cytoplasm and appear vesicle-like due to the absence of well-formed cristae in the inner mitochondrial membrane [80].

2.3. Genetic Profile

A number of major cardiac genes are expressed in hPSC-CMs at levels ~50% of adult human cardiac tissue [47]. In fact, hPSC-CMs are more similar to human fetal cardiac tissue than adult in terms of gene expression [36]. In addition to differences in expression levels, specific sarcomeric protein isoforms are associated with either fetal or adult CMs, e.g. β -myosin heavy chain (*MYH7*) and troponin I type 3 (*TNNI3*) are considered adult isoforms [47, 81, 82].

2.3.1. Genes associated with electrophysiology and calcium handling—The L-type voltage-gated Ca^{2+} channels are heterotetrameric complexes composed of α_1 , α_2/δ , β and γ subunits that allow fast influx of Ca^{2+} ions (I_{Ca} current) through the sarcolemma during the depolarization phase of the action potential [83] (Figure 3). The *CACNA1C* gene encodes the pore-forming α -subunit (α_{1C}) of the $\text{Ca}_v1.2$ voltage-gated Ca^{2+} channel, the predominant isoform present in the adult heart. In the embryonic myocardium, the $\text{Ca}_v1.3$ (α_{1D}) channel is the predominant isoform [83, 84]. Functionally, reduced expression of L-type Ca^{2+} channels has been associated with a non-existent or shortened plateau phase in the action potential of hESC-CMs [85].

The α -subunit of the $\text{Na}_v1.5$ voltage-gated Na^+ channel is encoded by the *SCN5A* gene. This α -subunit is the predominant cardiac isoform and allows a fast influx of Na^+ ions (I_{Na} current) through the sarcolemma during the depolarization phase of the action potential [86] (Figure 3). A slower depolarization velocity (V_{max}) in hPSC-CMs relative to adult CMs was attributed to reduced levels of $\text{Na}_v1.5$ [85].

The *KCND3* gene also encodes for an ion channel α -subunit, the $\text{K}_v4.3$ voltage-gated K^+ channel. The $\text{K}_v4.3$ channel contributes to the cardiac transient outward potassium current (I_{to}), the main current of early (phase 1) repolarization of the action potential [87] (Figure 3). Irregularities in the shape of the action potential in hPSC-CMs relative to adult CMs was attributed to an insufficiency of $\text{K}_v4.3$ [88].

The $\text{Kir}2.1$ voltage-gated K^+ channel contributes to the cardiac inward rectifier potassium current (I_{K1}), which functions in the late repolarization phase of the action potential, in stabilizing the diastolic potential and the resting membrane potential [89, 90] (Figure 3). The gene encoding the α -subunit of the $\text{Kir}2.1$ channel is *KCNJ2*. Acceleration of the diastolic depolarization phase and an elevated resting membrane potential in hPSC-CMs relative to adult CMs was attributed to an insufficiency of $\text{Kir}2.1$ [85, 91].

The $\text{Na}^+/\text{Ca}^{2+}$ -exchanger is the predominant mechanism of Ca^{2+} efflux and Na^+ influx during relaxation/repolarization (Figure 3). This ion transport protein also functions in maintaining the resting membrane potential. It is encoded for by the *SLC8A* gene.

The *ATP2A2* gene encodes for the sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA) isoform SERCA2. SERCA2 is the only active Ca^{2+} transporter in the SR and its splice variant SERCA2a is the predominant cardiac isoform. SERCA functions in both contraction and relaxation: it regulates the amount of SR Ca^{2+} that can be mobilised during contraction, and clears cytosolic Ca^{2+} to enable muscle relaxation [92].

The most abundant Ca^{2+} buffering protein in the lumen of the SR is calsequestrin and its cardiac isoform is encoded for by the *CASQ2* gene. It can bind Ca^{2+} with high affinity enabling calcium to be stored at total concentrations of 20mM, while free Ca^{2+} concentration remains at ~1mM [93].

The ryanodine receptor is a transmembrane Ca^{2+} channel in the SR that serves to release Ca^{2+} from the SR lumen into the cytosol. The predominant cardiac isoform of the ryanodine receptor is encoded for by the *RYR2* gene.

Connexin-43 is the major protein of gap junctions and is encoded by the *GJA1* gene.

2.3.2. Genes associated with contractile function—Cardiac muscle myosin is a hexamer of 2 heavy chains, 2 essential light chains and 2 regulatory light chains. The *MYH6* gene encodes for the (fast) α -heavy chain subunit of cardiac myosin (α -MHC) and the *MYH7* gene encodes for the (slow) β -heavy chain subunit of cardiac myosin (β -MHC). The β -MHC isoform predominates at all developmental stages, i.e. β -MHC/ α -MHC ratio >1 . Both isoforms are less abundant in the fetal heart than the adult [94] but the expression of α -MHC is higher in the fetal heart than the adult heart [95]. Thus, the β -MHC/ α -MHC ratio increases with developmental maturity. Relative to α -MHC, β -MHC has reduced ATPase activity and actin filament sliding velocity, but increased energy efficiency in generating contractile force [96–98]. Changes in the β -MHC/ α -MHC ratio therefore correlate with contraction velocity and heart rate [47]. Notably, a transition in myosin isoform content can be induced by disease or pathological stimulation, which complicates its utility in assessing maturity [99–101].

The (slow) ventricular isoform of the regulatory myosin light chain (MLC2v) is encoded for by the *MLY2* gene, whereas the *MLY7* gene encodes for the (fast) atrial isoform of the regulatory myosin light chain (MLC2a). MLC2 functions in the regulation of cardiac contractility [102]. In fetal human hearts, MLC2a is expressed in all chambers, whereas in adult hearts MLC2a expression is restricted to the atria [103]. In contrast, MLC2v expression is restricted to the ventricles throughout development and adulthood [103]. In human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs), MLC2a is the predominant isoform expressed at early developmental stages; at later time points, MLC2v is robustly expressed but in association with significant MLC2a expression [44].

The *ACTC1* gene encodes for the cardiac isoform of α -actin, a major constituent of the contractile apparatus. In the fetal and neonatal human heart, skeletal α -actin— encoded by the *ACTA1* gene— is the predominant isoform expressed whereas cardiac α -actin predominates in the adult heart [104].

Natriuretic peptides are secreted hormones implicated in extracellular fluid volume regulation and electrolyte homeostasis. The *NPPA* gene encodes atrial natriuretic peptide (ANP) and the *NPPB* gene encodes brain natriuretic peptide (BNP). In the normal adult heart, ANP and BNP are secreted by the atria and ventricles, respectively, primarily in response to increased stretch of the myocardium [105]. In addition to volume regulation, ANP modulates cell growth and proliferation— e.g. inhibits vascular smooth muscle cell proliferation [106] and inhibits hypertrophy [107] — and both ANP and BNP inhibit cardiac fibroblast growth [108]. The levels and localization of ANP and BNP expression is developmentally regulated. In the fetal heart, the ANP expression in the ventricles is 10-fold higher than in the adult ventricles [94], and decreases with gestational age [109–111]. In the adult heart, the atria are the major site of ANP expression [112]. Similarly, BNP expression in fetal ventricles has been reported to be greater than in the adult ventricle [113–115]. Notably, ANP and BNP expression is altered in various disease states, and are used as markers of pathological hypertrophy [116].

Troponin is a heterotrimeric protein complex composed of troponin-I, troponin-C and troponin-T that regulates the actin-myosin interaction by changing the position of tropomyosin on the actin-based thin filaments in response to changes in cytosolic Ca^{2+} [117]. Troponin-I is the inhibitory subunit of the troponin complex. The fetal heart expresses the *TNNI1* gene encoding the (slow) skeletal isoform of troponin-I. Shortly after birth this isoform is completely and irreversibly replaced by the adult cardiac isoform of troponin-I, encoded by the *TNNI3* gene. The interaction of troponin-I with troponin-C and troponin-T, and with actin-tropomyosin is different depending on the isoform. Functionally, the isoforms differ in Ca^{2+} -binding kinetics and cardiac muscle relaxation rates [118].

The *TNNT2* gene encodes for the cardiac isoform of troponin-T, the tropomyosin-binding subunit of the troponin complex. Importantly, cardiac troponin-T is also expressed in non-cardiac cells such as smooth muscle cells [5, 60, 119–122], which limits its utility as a marker of the cardiac lineage.

Myosin binding protein C is a thick-filament (myosin)-associated protein localized to the A-bands of sarcomeres. The cardiac isoform is encoded by the *MYBPC3* gene. Myosin binding protein C functions in the regulation of contraction by regulating the activity of actomyosin ATPase [123].

The *ACTN2* gene encodes for $\alpha 2$ -actinin, an actin-binding protein localized in the Z-disc. Its function is to anchor the actin filaments.

Titin— encoded by the *TTN* gene— is a large sarcomeric filament that extends from the Z-disk to the M-band in the sarcomere [124] and contributes to passive tension [125–129]. The expression of different splice variants with different spring constants is developmentally-regulated. In the fetal and neonatal heart, a very long isoform with additional spring elements predominates (fetal cardiac titin, FCT) [130–132]. During postnatal development, expression switches from the FCT isoform to the adult isoforms: a long relatively compliant isoform (N2BA) and a shorter stiffer isoform (N2A) [133–135]. In the adult left ventricle, the N2BA/N2B ratio is ~ 0.6 [134]. Functionally, passive tension increases with developmental age owing to the switch from the compliant FCT isoform to the stiffer N2BA/N2B isoforms.

2.4. Functional Properties

In cardiac excitation-contraction coupling, excitation (membrane depolarization) stimulates the movement of Ca^{2+} to activate muscle contraction. During depolarisation, Ca^{2+} enters the cell through L-type voltage-gated Ca^{2+} channels in the sarcolemma and activates nearby Ca^{2+} -dependent ryanodine receptors in the SR. Ryanodine receptor activation initiates the coordinated release of Ca^{2+} from the SR, i.e. calcium-induced calcium release [136]. Calcium-induced calcium release yields a rapid increase in intracellular Ca^{2+} from 100nM to 1 μ M [137]. This transitory global increase in cytosolic Ca^{2+} , i.e. Ca^{2+} transient, provides sufficient Ca^{2+} to saturate troponin-C [138]. This leads to a change in the troponin/tropomyosin complex configuration that removes the troponin-I-mediated inhibition and allows the interaction of actin with myosin. Actin-myosin cross-bridge cycling induces the sarcomere to shorten and the muscle to contract [79]. Reduction in cytosolic Ca^{2+} by the

sarcolemmal $\text{Na}^+/\text{Ca}^{2+}$ exchanger transporting Ca^{2+} out of the cell and SERCA actively pumping Ca^{2+} into the SR, dissociates Ca^{2+} from troponin-C, reinstating troponin-I inhibition and muscle relaxation [139].

2.4.1. Electrophysiology and calcium handling—The adult CM action potential requires the precisely orchestrated activity of several ion channels, the expression and function of which are developmentally-regulated. CMs undergo changes in electrical properties during fetal and postnatal heart development due to developmental changes in ion channels. These changes ultimately lead to the acquisition and maintenance of a mature cardiac electrophysiological phenotype. It is well established that hPSC-CMs are electrophysiologically immature, expressing the major ion currents at levels that are fetal-like rather than adult-like [38, 140].

In adult CMs, calcium-induced calcium release from the SR contributes almost 70% of the total Ca^{2+} release [141]. In contrast, hPSC-CMs have very little SR function in the early phase [66, 142–146] akin to fetal CMs, which have structurally and functionally underdeveloped SRs with decreased volume and limited capacity to load Ca^{2+} [147, 148]. As a consequence, hPSC-CMs demonstrate smaller Ca^{2+} transients [149] that occur primarily by the slower process of Ca^{2+} influx across the cell membrane and its subsequent diffusion through the cytoplasm [69]. This results in abnormal diffusion of Ca^{2+} in the cell [145], which causes non-uniform Ca^{2+} dynamics across the cell— greater Ca^{2+} transient peak amplitude in the cell periphery than the centre [47] — and reduces the contractile synchronicity necessary for the generation of large contractile forces [29, 38]. While reports vary as to the presence and function of the SR in hPSC-CMs, possibly due to maturation differences among the study populations [66, 143, 144, 150, 151], there is a consensus that intracellular Ca^{2+} stores are smaller in hPSC-CMs than in adult CMs [66, 152].

The depolarization velocity reported for hPSC-CMs [37, 53, 153] was 6- to 50-fold lower than the V_{max} reported for human adult cardiac tissue [154] but within the same range as reported for fetal ventricular CMs [37]. Long-term culture (> 120 days) of hPSC-CMs was demonstrated to increase V_{max} to within 25% of the adult V_{max} [60] (Table 1).

The action potential amplitude (APA) reported for hPSC-CMs was within the same range as for human adult cardiac tissue (76–116mV [37, 53, 153] and 102–110mV [154], respectively) and 3- to 6-fold higher than reported for fetal ventricular CMs [37] (Table 1).

Maximal diastolic potential refers to the most negative transmembrane potential achieved by a cardiac cell during repolarization. The maximal diastolic potential reported for hPSC-CMs was 13–53% higher than reported for human adult cardiac tissue [153, 154] (Table 1).

The resting membrane potential reported for hPSC-CMs was 30–50% higher than reported for adult human CMs [37, 60, 155] but within the range reported for fetal CMs [37, 156]. Long-term culture (> 120 days) of hPSC-CMs did not significantly decrease the RMP (30–40% of adult RMP) [60] (Table 1).

The reported action potential duration (APD) for hPSC-CMs vary widely from 200–500ms [23, 37, 53, 153, 157], i.e. from duration times on par with those reported for adult human

cardiac tissue (228–259ms [154]) to nearly double the adult APD. The APD for fetal ventricular CMs was 25–80% longer than for adult (324–416ms [37]) (Table 1).

Cell membrane capacitance describes the ability of the membrane to retain its charge, i.e. the amount of charge required to change the membrane potential. The reported capacitance for hPSC-CMs [158] was 8- to 10-fold lower than for adult human ventricular CMs [155, 159, 160] (Table 1).

Conduction velocity is the speed at which an action potential can be transmitted through the tissue. For adult human cardiac tissue, the reported conduction velocity had a wide range (30–100cm/s) [47], 2- to 100-fold faster than reported for hPSC-CMs (1–15cm/s) [23, 157]. The reported conduction velocity for intact human fetal hearts was within the range of adult values (40–70cm/s) [156] (Table 1). Conduction velocity is developmentally regulated. It is dependent upon gap junctional coupling, I_{Na} —which depends on Na^+ conductance and the resting membrane potential—and cell size, properties that change during cardiac development [161].

APD (electrical) restitution describes the decrease in APD as a consequence of increased heart rate [162–166]. Its function is to enable more time for coronary perfusion and ventricular filling at short cycle lengths. It has been suggested that a shorter diastolic interval results in a shorter APD because ion channels have decreased recovery time. APD restitution curves can be used to assess electrical instability particularly at high pacing rates. This is because when the curve is steep (slope > 1) there is an increased susceptibility to alternating beat-to-beat changes in APD (APD alternans) [166], an arrhythmogenic scenario [162]. APD restitution has been demonstrated in both adult and fetal human hearts [156, 164, 167].

Analogous to the APD restitution curve, conduction velocity restitution describes the rate-dependence of conduction velocity [162]. At high beat rates, the diastolic interval is not long enough for Na^+ channels to recover from inactivation, which results in a decrease in the conduction velocity [162, 168]. The conduction velocity restitution curve can also be used to assess electrical instability. Conduction velocity restitution is suggested to function in the conversion of spatially concordant APD alternans to spatially discordant APD alternans when the curve is steep (slope > 1) [168].

The decay constant is used to quantify the rate of recovery of intracellular Ca^{2+} to resting levels. It describes the time required for the Ca^{2+} transient amplitude or AP amplitude to decrease 37% and is calculated by an exponential fit to the decay portion of the Ca^{2+} transient curve [169, 170]. The decay constant is dependent on the rate of Ca^{2+} efflux from the cell, which is dependent upon the membrane resistance, i.e. the number of open ion channels, and on membrane capacitance [171].

2.4.2. Contractile stress—Spontaneous beating and synchronous contraction (on a macroscopic level) has been demonstrated for hPSC-CMs soon after differentiation has been initiated [23]. However as mentioned previously, the abnormal Ca^{2+} handling in the immature CM results in non-uniform Ca^{2+} dynamics across the cell [47, 145], which

prevents global synchronous contraction and hence large force generation [29, 38]. Additionally, the expression of contractile and cytoskeletal genes is much lower in hPSC-CMs as compared with fetal or adult CMs [95, 172]. Taken together, it is not surprising that the contractile force generated by hPSC-CMs is significantly lower than in adult cardiac tissue [47, 173].

Adult ventricular CMs require an external stimulus in order to beat [174], whereas, hPSC-CMs and hECTs beat spontaneously. The spontaneous beat rate of hPSC-CMs has been reported to range from 21 to 84 beats per minute [73, 153, 175–178]. This spontaneous activity is present in CMs during early embryonic stage but is supplanted by a quiescent cell phenotype as development progresses [161].

Synchronous contractions are often used to indicate functional coupling of hESC-CMs [18, 179]. However, the amount of coupling conductance required for synchronization may be significantly lower than the adult and/or fetal requirement of 3–12 μ S [180].

Contractile stress, the force per unit area developed by the myocardial fibers, is developmentally-regulated, such that the adult myocardium develops more force than fetal myocardium [181]. The adult CMs are also able to shorten further and faster than immature CMs [182]. The active stress generated by freshly isolated human ventricular CMs was >50 mN/mm² [183], whereas isolated ventricular tissue from neonatal (<2week) hearts generated a contractile stress of 0.8–1.7 mN/mm² [184]. When measured under similar substrate conditions, the contractile stress of seeded fetal (14–19 week) CMs was ~0.4mN/mm² [53] and 0.15–0.30mN/mm² for hPSC-CMs [185, 186].

In the adult heart, increased venous return to the heart results in increased fibre length, which produces increased contractile force and stroke volume: the Frank-Starling mechanism. This mechanism has been attributed to an increase in Ca²⁺ sensitivity at longer sarcomere lengths that induces an increase in the force of contraction [79, 187]. The Frank-Starling mechanism is apparent in human fetal heart after 10–15 week of gestation [188]. In ECTs where stroke volume cannot be assessed, the Frank-Starling mechanism is approximated by isometric force measurements at different tissue lengths, i.e. % tissue elongation. Increasing force with increasing length is considered a positive Frank-Starling relationship.

Adult human CMs have a positive force-frequency relationship, i.e. Ca²⁺ transients and force of contraction increase with increasing pacing rates [141]. Neonatal myocardial tissue has a flat or slightly negative force-frequency relationship that becomes increasingly positive with age [184]; and hPSC-CMs frequently display negative force-frequency relationships [150, 152, 189]. A positive force-frequency relationship requires significant store of intracellular Ca²⁺ and electrical coordination across the cell, which is associated with the presence of T-tubules [141].

Excitation threshold (ET) is the minimum electrical field strength, or the pulse intensity of point stimulation, required to stimulate the tissue to contract simultaneously, and is used as a quantitative measure of cardiac electrical function [18].

The maximum stimulation frequency that can induce an hECT to beat simultaneously is the maximum capture rate (MCR). It is used to quantitatively measure electrical function because it is dependent upon the expression and kinetics of repolarization and depolarization currents, and on effective gap junction coupling [161].

2.5. Pharmacological response

The accuracy with which a test compound can be screened or validated in an ECT is dependent upon how closely the *in vitro* model approximates the target tissue or organ. In cardiac tissue engineering, it has become apparent that the developmental stage of the hESC-CMs affects the sensitivity and response to various pharmacological agents [190]. A comparison of the pharmacological responses of human adult and fetal myocardium/CMs, as well as hPSC-CMs to the compounds described below is provided in Table 5.

An important aspect of heart tissue maturation is the inotropic response to extracellular Ca^{2+} . The half-maximal effective concentration (EC_{50}) for extracellular Ca^{2+} in adult muscle strips was 3.0mM [191], whereas hPSC-CMs display hypersensitivity toward external Ca^{2+} with an EC_{50} of 0.4–1.8mM [192, 193].

The response to β -adrenergic receptor stimulation by e.g. epinephrine, norepinephrine or isoproterenol, is used as a measure of maturity because the effects that are characteristic of the adult myocardium—positive inotropic, chronotropic and lusitropic effects [84, 194]—require a functional SR to enable the rapid release of increased amounts of Ca^{2+} [143, 195]. Activation of the β -adrenergic receptors initiates an intracellular signalling cascade that results in the phosphorylation of $\text{Ca}_v1.2$, troponin-I, and phospholamban (Figure 4). Phosphorylation of the L-type Ca^{2+} channel $\text{Ca}_v1.2$ causes a ~3-fold increase in I_{Ca} in CMs [84]. Phosphorylation of troponin-I increases the actin-myosin cross-bridge cycling rate by altering the Ca^{2+} -binding kinetics of troponin-C, the Ca^{2+} -binding subunit of the troponin complex [194, 196]. Phosphorylation of phospholamban, a regulatory protein that reduces the affinity of SERCA2a for Ca^{2+} [197], increases the velocity of Ca^{2+} transport by SR vesicles, which leads to an increased rate of SR Ca^{2+} re-uptake (increased relaxation velocity) and SR Ca^{2+} loading, such that there is increased SR Ca^{2+} release in subsequent beats (increased peak force) [79, 194, 196].

Epinephrine (adrenaline) and norepinephrine (noradrenaline) are natural catecholamines, and isoproterenol (isoprenaline) is a synthetic catecholamine, i.e. β -adrenergic receptor agonists. In human adult papillary muscles, norepinephrine was demonstrated to induce a positive inotropic and chronotropic response. In hESC-CMs, the positive inotropic response is often less pronounced than in adult hearts [198], and frequently a chronotropic response is observed in the absence of an inotropic response [29, 193]. For isoproterenol, an EC_{50} of 12.9nM was determined for hESC-CMs based on the chronotropic response [175], whereas an EC_{50} of 11–80nM was reported for adult human myocardium based on the inotropic response [199–202].

Carbachol is an agonist of the parasympathetic nervous system [175]. Cholinergic stimulation induces a negative chronotropic, dromotropic and inotropic response in adult human cardiac preparations [203–205]. An EC_{50} of 140nM was determined for isolated

human ventricular papillary muscle strips [206]. In hESC-CMs and human fetal ventricular cells, carbachol was demonstrated to have similar negative chronotropic effects [37].

Verapamil inhibits the $K_v11.1$ (human ether-a-go-go, hERG) and $Ca_v1.2$ channel at overlapping concentrations and has been observed to induce a negative dromotropic, chronotropic and inotropic effect. For the hERG current, a half-maximal inhibitory concentration (IC_{50}) of 143 nM has been reported [207, 208]; and for the $Ca_v1.2$ channel, an IC_{50} of 0.24–4.3 μ M has been reported [209–211] based on cell lines stably-transfected to express the respective human channel. In adult cardiac tissue (right atrial) an IC_{50} of 123.0 nM was reported based on the inotropic response subsequent to isoproterenol treatment [212]. In fetal and hESC-CMs, verapamil inhibited AP propagation, however fetal CMs were more responsive to verapamil than hESC-CMs [37]. Verapamil treatment also induced a positive chronotropic effect in hiPSC-CMs [208, 213]. The positive chronotropic effect of L-type Ca^{2+} channel blockers in hiPSC-CMs has been suggested to be indicative of differences in Ca^{2+} handling in neonatal and adult CMs; and mechanistically may be due to decreased APD and increased diastolic interval enabling increased action potential frequency [208, 213].

Nifedipine is an L-type Ca^{2+} channel antagonist. Nifedipine inhibited the $Ca_v1.2$ channel with an IC_{50} of 16–24 nM [210] based on cell lines stably-transfected to express the human channel. A dose-dependent decrease in APD has been observed with nifedipine treatment in hPSC-CMs, as well as a negative inotropic response and a positive chronotropic response [208, 213]. An IC_{50} of 39 nM has been reported for hiPSC-CMs [214].

Nisoldipine is also an L-type Ca^{2+} channel antagonist, specific for $Ca_v1.2$ with an IC_{50} of 67–81 nM based on cell lines stably-transfected to express the human channel [215]. Inhibition of AP propagation and contraction was demonstrated with 1 μ M nisoldipine treatment of hESC-CM EBs [43].

Thapsigargin is used to determine SR functionality [216]. It is an inhibitor of SERCA, the primary Ca^{2+} reuptake pump in the SR and therefore causes the intracellular Ca^{2+} stores to be depleted. In adult CMs, a strong negative inotropic effect results from the depletion of SR Ca^{2+} stores [217] because SERCA accounts for 70% of Ca^{2+} reuptake from the cytosol back into the SR [141]. In hESC-CMs, treatment with 100 nM thapsigargin did not affect the Ca^{2+} transient or contraction [143], whereas treatment with 500 nM [216] and 1 μ M thapsigargin did induce a significant decrease in Ca^{2+} transient amplitude [218]. Treatment with 10 μ M thapsigargin was demonstrated to decrease Ca^{2+} transient amplitude in both hESC-CMs and hiPSC-CMs, and a dose-dependent decrease in Ca^{2+} transient amplitude (40–100%) was demonstrated in hiPSC-CMs with 1–20 μ M thapsigargin [144].

Caffeine is also used to determine SR functionality [216]. Caffeine is an agonist of the ryanodine family of receptors, the primary Ca^{2+} release channels in the SR responsible for excitation-contraction coupling [219]. Some preparations of hESC-CMs have been demonstrated to be insensitive to treatment with 10 mM caffeine [143], while others have been shown to respond to increasing caffeine concentrations (10–40 mM) with a dose-dependent increase in Ca^{2+} transient amplitude [144]. Relative to fetal ventricular CMs, it

was demonstrated that the increase in Ca^{2+} transient amplitude induced by 20mM caffeine was significantly lower in hESC-CMs [218]. Conversely, it has also been reported that the increase in Ca^{2+} transient amplitude induced by 10mM caffeine treatment was the same in hESC-CMs and fetal ventricular CMs but the fraction of responsive cells was lower among hESC-CM preparations relative to fetal CMs [216].

E-4031 is an inhibitor of the $\text{K}_{\text{v}}11.1$ (hERG), which mediates the repolarizing I_{Kr} current in the action potential [220]. Inhibition of I_{Kr} results in a prolongation of the time between depolarization and repolarization [221], hence E-4031 affects APD. An IC_{50} of 7–32nM was reported based on cell lines stably-transfected to express the human channel [208, 211]. hPSC-CMs have been demonstrated to be responsive to E-4031 with an IC_{50} of 17nM [208, 214].

Terfenadine is an antihistamine that was withdrawn from the market due to unacceptable risk of arrhythmia. Terfenadine blocks K^+ , Na^+ , and Ca^{2+} cardiac ion channels [220, 222]. In isolated human atrial CMs, terfenadine inhibited $\text{Na}_{\text{v}}1.5$ with an IC_{50} of 1.7–8.1 μM [223] and inhibited L-type Ca^{2+} currents with an IC_{50} of 185 nM [224]. Terfenadine inhibited the hERG current with an IC_{50} of 56 nM based on cell lines stably-transfected to express the hERG channel [225], and 26nM in isolated human atrial CMs [223]. Terfenadine treatment was demonstrated to prolong APD in hPSC-CMs at >10nM; and at 1 μM a negative inotropic effect was observed [214].

Tetrodotoxin is a Na^+ channel ($\text{Na}_{\text{v}}1.5$) blocker isolated from puffer fish toxin, which has been demonstrated to have a negative chronotropic response [226, 227], with an IC_{50} 1 μM [228, 229]. In human cardiac papillary muscle preparations, tetrodotoxin treatment prolonged the APD and slowed V_{max} [230].

Lidocaine is a Na^+ channel inhibitor with an IC_{50} of 34–38 μM based on a stably-transfected cell line expressing human Na^+ current [231]. Lidocaine treatment prolonged the APD and slowed V_{max} in human ventricular muscle preparations [232]. In hiPSC-CMs, lidocaine blocked Na^+ influx and decreased conduction velocity but did not affect the APD [233].

3. Human Engineered Cardiac Tissue

Three-dimensional ECTs have yielded great success in maturing hPSC-CMs. Three-dimensional microenvironments have been demonstrated to affect the gene expression and function of the embedded cells, independent of soluble factors [234]. A variety of physiological parameters have been demonstrated to affect hPSC-CM maturation including: long-term culture [60], substrate stiffness [235], cell patterning and alignment [45, 236], electrical and mechanical stimulation [237], mechanical loading [238] and the interaction with other cell types [239]. The majority of work to date in developing suitable 3D environments for cardiac cell cultivation, has been performed using mouse, rat and chick CMs but due to recent progress in the stem cell field, an abundant human CM source is now available. As such, in the last 5 years, cardiac tissue engineering has begun to move away from animal-based ECTs, translating the acquired knowledge into the production of human ECTs. Human ECTs (hECTs) provide an important species-specific advantage over animal models in the development of new therapeutics, as well as in providing species-specific

information about cardiogenesis. Additionally, because CMs derived from biopsies are considered terminally differentiated and thus cannot supply large numbers of CMs for therapeutic and basic science investigations, the use of hPSC-CMs in hECTs enables the production of large numbers of hECTs for the first time. The following section and Tables 2–4 review the hECTs that have been developed.

3.1. Static and cyclic stretch

Utilizing the understanding that mechanical conditioning can induce maturation, Tulloch *et al* [32] generated a 3D hECT from hPSC-CMs in a collagen/Geltrex gel seeded into a 20mm x 3mm channel with a deformable silicon floor and nylon mesh anchors (FlexCell Tissue Train). A subset of hECTs included human umbilical vein endothelial cells (HUVECs) and human marrow stromal cells (hMSCs) or mouse embryonic fibroblasts (MEFs) in the initial cell population. Upon tissue compaction, the hECT was suspended across the channel anchored at both ends by the nylon mesh, and therefore subjected to static stretch. The hECTs were cultured for between 4 and 21 days. After 5 days in culture, the hECTs had uniform CM distribution and relative to the unstretched tissue (anchored at only one end), static stretch increased cell and collagen alignment, and increased the average CM size and number. After 7 days of culture, myofibril and Z-disc alignment increased and some polyribosomes were observed to be associated with the myofilaments. Functionally, the hECTs beat synchronously after 5 days, and after 21 days of static stretch, the hECTs were demonstrated to generate a Frank-Starling curve.

A subset of hECTs were conditioned with 4 days of uniaxial cyclic stretch (5% elongation at 1Hz). Relative to the statically-stretched hECT, cyclic stretch increased the average CM size and increased the number of proliferative CMs. Genetically, cyclic stretch upregulated *MYH7* (β -MHC), *TNNT* (cTnT), *NPPA* (ANP), *NPPB* (BNP), *CACNA1C* (L-type calcium channel subunit 1C α), *RYR2* (sarcoplasmic calcium channel/ryanodine receptor) and *ATP2A2* (SERCA2); and increased the spontaneous beat rate.

The addition of HUVECs to the hECTs did not affect CM or collagen alignment but did increase the percentage of proliferative CMs. Moreover, the addition of HUVECs introduced cord and luminal vessel structures into the hECTs, and the addition of stromal cells (hMSCs or MEFs) increased the total number of structures 8- to 10-fold. To demonstrate the utility of these hECTs in regenerative therapy, statically-stretched hECTs containing CMs only or CMs in combination with HUVECs and MSCs were implanted onto the uninjured epicardium of athymic rat hearts. After 1 week, the hearts were harvested and juxtaposition of the hECT and host myocardium was demonstrated in the absence of a foreign body response. The hECTs were also shown to retain both sarcomeric organization and cell alignment *in vivo*. For the hECTs composed of CMs, HUVECs and MSCs, anastomosis between the hECT and host vasculature was also demonstrated.

Deviating from the paradigm of using dissociated single cell hPSC-CMs, Kensah *et al* [34] produced hECTs from pure hPSC-CM cardiac bodies obtained from embryoid body (EB) differentiation of transgenic hPSC lines expressing an antibiotic-resistance gene under the control of the cardiac-specific *MYH6* promoter. The hECTs were prepared by seeding cardiac bodies with fibroblasts in a collagen/Matrigel gel into wells with titanium rods at

either end positioned 6mm apart. Upon tissue compaction, the resulting hECT was suspended between the two titanium rods and thus subjected to static stretch conditioning. From preliminary experiments with a transgenic murine iPSC line, it was determined that whole cardiac bodies could be used to form mouse ECTs (mECTs), which eliminated the need for dissociation and thereby prevented the disruption of cell-cell interactions and dissociation-associated cell death. It was demonstrated that the formation of uniformly distributed, compact and viable mECTs capable of simultaneous and synchronous contraction, and increased maximum active force required the addition of 10% fibroblasts to the input population. Furthermore, it was shown that stepwise growth stretch—incremental increases in tissue length over the course of culture period—yielded mECTs with more uniform distribution of viable, metabolically-active CMs, increased maximum active and passive force, increased CM alignment, increased sarcomeric length and alignment than either static stretch or cyclic stretch (10% elongation at 1Hz). Finally, the preliminary experiments demonstrated that ascorbic acid supplementation increased sarcomeric organization, increased CM alignment, resulted in better developed intercalated discs, increased collagen and titin production, increased maximum active force and passive force, and nuclei per area.

Based on these findings, hECTs were generated from hPSC-derived cardiac bodies and fibroblasts, were cultured in the presence of ascorbic acid and were mechanically conditioned by growth stretch, i.e. stretched at 400µm-increments every 3–4 days from day 7 to day 21. Morphologically, day 21 hECTs had longitudinally aligned CMs and collagen bundles, a high density of viable CMs, organized and aligned sarcomeres, and after 4 weeks in culture, 2.5–4.2% of CMs were proliferative. Connexin-43 was found to be expressed but at levels significantly lower than in native heart and localized at lateral CM borders. Genetically, *MYL2* (MLC2v) expression was upregulated to adult levels after 22 days of culture; and the MLC2v/MLC2a ratio significantly increased in a time-dependent manner. *KCNJ2* (Kir2.1) expression also significantly increased with time and *NPPA* (ANP) expression was significantly downregulated relative to cardiac bodies after 7 days of culture. Functionally, day 21 hECTs spontaneously beat and efficient excitation-contraction coupling was demonstrated with a maximal conduction velocity of 4.9cm/s. A positive Frank-Starling relationship was demonstrated for both active force and passive force, and on day 21 maximum active force was measured at 1.37 ± 0.18 mN and passive force at 4.66 ± 0.64 mN. Pharmacologically, a positive chronotropic and inotropic response was demonstrated with 100nM isoproterenol, which was reversed by subsequent treatment with 10µM carbachol.

Focusing specifically on the effects of cyclic stretch on hESC-CM maturation, Mihic *et al* [238] generated hECTs from hESC-CMs in a 30mm x 10mm x 7mm gelatin sponge. After 2 days of compaction, a subset of hECTs were subjected to uniaxial cyclic stretch (12% elongation at 1.25Hz) for 3 days in a custom reactor. Cyclically-stretched hECTs had more even cell distribution at greater depths; fewer cell aggregates; increased cell size and elongation; increased number of CMs; increased number of well-defined, aligned and organized Z-discs; increased connexin-43 expression and increased gap junctions at lateral CM borders, relative to unstretched controls. Cyclic stretch also increased the diffusion of

oxygen and nutrients. Genetically, the expression of *CACNA1C* ($\text{Ca}_v1.2$), *KCNH2* ($\text{K}_v11.1$), *SCN5A* ($\text{Na}_v1.5$) and *KCNJ2* ($\text{Kir}2.1$) were significantly upregulated in cyclically-stretched hECTs relative to unstretched hECTs. Cyclic stretching induced upregulation of *MHY7* (β -MHC) and *NPPA* (ANP), and downregulation of the early development transcription factor *MEF-2C* (myocyte-specific enhancer 2C). Cyclic stretching also induced increased protein expression of MLC2v and cardiac α -actin, and decreased protein expression of MEF-2c and ANP. Functionally, the hECTs were demonstrated to have electrically coupled Ca^{2+} transients, i.e. contraction observed to occur near simultaneously with peak Ca^{2+} transients. The cyclically-stretched hECTs cycled Ca^{2+} faster (70.5 ± 7.1 cycles/min), had shorter Ca^{2+} cycle durations (0.50 ± 0.07 s) than the unstretched hECT. To demonstrate the utility of these hECTs in regenerative therapy, unstretched and cyclically-stretched hECTs and acellular scaffolds ($\sim 7.0 \times 8.0 \times 3.5$ mm) were surgically implanted into a rat I/R model over the injured epicardium. After 2 weeks, electrical coupling between the graft and host myocardium was demonstrated. The AP amplitude for the graft was smaller than for the host myocardium, but the $\text{APA}_{\text{host}}/\text{APA}_{\text{graft}}$ was significantly closer to unity for cyclically-stretched hECTs than unstretched hECTs or acellular scaffolds. There was also significantly less anterior wall thinning with the stretched hECTs relative to the unstretched hECTs. Upon heart excision, it was demonstrated that the stretched graft was larger than either control and contained a high density of elongated cells with evident striations up to 500 μm from the attached surface.

The pioneering work of Eschenhagen, Zimmermann and colleagues [240] was transitioned from the production of rat ECTs to human ECTs by Streckfuss-Bömeke *et al* [192]. A ring-shaped hECT was generated from hPSC-CMs in a collagen/Matrigel gel seeded into circular molds. After 3 days of compaction, the ring-shaped hECTs were mechanically conditioned by uniaxial cyclic stretch (10% elongation at 2Hz) for 7 days in a custom reactor. After 10 days of culture, the hECTs were characterized by densely packed, aligned muscle bundles with organized and aligned sarcomeres. The hECTs beat spontaneously and generated a Frank-Starling curve. Pharmacologically, a positive inotropic response to extracellular Ca^{2+} ($\text{EC}_{50} = 0.4\text{mM}$) and isoproterenol (1 μM) was demonstrated.

In an effort to design a hECT platform for testing the proarrhythmic effects of drugs, Schaaf *et al* [43] generated hECTs from hESC-CMs in a fibrin/Matrigel gel seeded into a 12mm x 3mm x 3mm agarose casting mold in which 2 elastic silicone posts were inserted from above. The hECTs were cultured for 5 weeks. Upon compaction, the hECTs were suspended between the 2 flexible posts, thus subjected to static stretch. Morphologically, hECTs had significantly increased CM alignment compared with age-matched EBs, well-developed sarcomeres that were evenly distributed throughout the cell (nuclei and periphery), and expressed connexin-43 although not in an intercalated disc-localized pattern (Figure 1D). Genetically, *MYH7* (β -MHC) and *NPPA* (ANP) expression increased in hECTs relative to EBs, after 5 weeks in culture. Functionally, the hECTs were demonstrated to contract and deflect the posts after 5–10 days of culture. After 5 weeks, hECTs demonstrated excitation-contraction coupling and APD restitution, but exhibited discontinuous AP firing patterns. APD was demonstrated to be highly variable within hECTs ranging from 260–1200ms, with longer APDs associated with increased V_{max} . Pharmacologically, a positive chronotropic

and inotropic response to increasing concentrations of extracellular Ca^{2+} was demonstrated for hECTs, with an EC_{50} of $\sim 0.8\text{mM}$ for the chronotropic response and $\sim 1.0\text{mM}$ for the isotropic response. A positive chronotropic and inotropic response to 100nM isoproterenol was also demonstrated; and subsequent treatment with $10\mu\text{M}$ carbachol decreased the beat rate by $\sim 50\%$ but had no effect on force. The response to $1\mu\text{M}$ nisoldipine—cessation of AP propagation and contraction; to $3\mu\text{M}$ tetrodotoxin—decreased V_{max} , prolonged APD and diastolic interval; and to 300nM E-4031—significant prolongation of APD—was demonstrated to be similar for hECTs, age-matched EBs, and the initial seeding population. The hECTs were further tested with a series of proarrhythmic drugs: E-4031, procainamide, cisapride, quinidine, sertindole. Cisapride ($0.3\text{--}100\text{nM}$) induced a concentration-dependent decrease in contraction force, contraction velocity, relaxation velocity, and at highest concentration induced a decrease in beat rate. Sertindole ($1\text{--}300\text{nM}$) induced a concentration-dependent decrease in relaxation velocity, and at the highest concentration decreased the contraction force and contraction velocity. Quinidine ($10\text{nM}\text{--}1\mu\text{M}$) induced a concentration-dependent decrease in relaxation velocity, and at the highest concentration decreased the beat rate, contraction force and contraction velocity. Procainamide ($1\text{--}300\mu\text{M}$) induced a concentration-dependent decrease in beat rate, contraction force, contraction velocity and relaxation velocity. E-4031 ($1\text{--}30\text{nM}$) induced a concentration-dependent decrease in contraction force, contraction velocity, relaxation velocity and relaxation time, and at the highest concentration induced a decrease in beat rate and contraction time. An irregular beating pattern was observed at the highest concentrations for all proarrhythmic drugs.

The effect of static stretch on hESC-CM maturation was additionally investigated by Turnbull *et al* [193] generated 3D hECTs from hESC-CMs in a collagen/Matrigel gel seeded into a rectangular PDMS well with integrated posts positioned 10mm apart. Upon compaction, the self-assembled hECT was suspended between the 2 flexible posts, i.e. subjected to static stretch. The hECTs were cultured for 18–30 days. Morphologically, hECTs had densely packed centres with aligned CMs and collagenous tissue edges heavily populated with stromal cells, after 18–23 days of culture. The hECTs also had aligned myofibrils with distinct and organized Z-discs and mitochondria with cristae, but the SR were not longitudinally oriented. Connexin-43 was expressed but distributed along the lateral CM borders. Genetically, hECTs had *MYH6* (α -MHC) expression that was $\sim 40\%$, *MYH7* (β -MHC) that was $\sim 5\%$, *ATP2A2* (SERCA2a) that was $\sim 60\%$, and *ACTC1* (cardiac α -actin) that was 100% of adult levels, after 3 weeks in culture. Both *MYH7* and *ATP2A2* expression were demonstrated to increase with culture time, whereas *NPPA* (ANP) expression decreased with culture time but was still significantly elevated relative to adult levels. Functionally, hECTs were demonstrated to beat spontaneously and after 5–10 days of culture, the contraction force stabilized. The force generated by the hECTs was found to be $0.50\text{--}0.64\text{mN/mm}^2$, and the contraction and relaxation time of the hECTs were $\sim 50\%$ of the neonatal or infant values. The hECTs were responsive to electrical stimulation ($\text{ET} = 128 \pm 90\text{ mV/mm}$; $\text{MCR} = 3\text{ Hz}$) for at least 30 days. After 18–24 days in culture, the hECTs generated a Frank-Starling curve; a negative force-frequency relationship; and an inverse relationship between twitch duration and frequency. APD restitution was also demonstrated for hECTs. Pharmacologically, a positive inotropic response to increasing concentrations of

extracellular Ca^{2+} (0.5–2.5mM) was demonstrated for hECTs, with an EC_{50} of 1.8mM. The hECTs were demonstrated to have a negative inotropic response to increasing concentrations verapamil (0.001–10 μM), with an IC_{50} of 0.61 μM . Treatment with 1 μM verapamil induced a decrease in peak force as well as a decrease in contraction and relaxation time, and contraction and relaxation velocity. A positive chronotropic and a transient positive inotropic response to increasing concentration (0.001–10 μM) of isoproterenol was demonstrated for hECTs with an EC_{50} of 750nM based on the inotropic response. Treatment with 1 μM isoproterenol induced a significant decrease in contraction time, and non-significant decrease in peak force, relaxation time, and both contraction and relaxation velocity.

The ratio of non-myocytes to CMs used for cell seeding was investigated in detail in a study by Thavandiran *et al* [234]. They generated hECTs from hESC-CMs and hESC-derived cardiac fibroblasts in a collagen/Matrigel gel seeded into PDMS microwells with integrated posts. In comparing two microwell design variants—posts at either end of an elongated microwell to induce uniaxial stress or posts around the edge of a square well to induce biaxial stress—it was determined that uniaxial mechanical stress generated hECTs with highly aligned sarcomeres, collagen fibrils, and elongated and longitudinally-oriented cells. By adjusting the fibroblasts content of the hECTs it was determined that the optimal hECT composition was 75% CMs and 25% FBs based on the uniform dispersion of both cells types, synchronous contractions, efficient ECM remodeling and high tissue integrity. The hECTs were therefore generated in elongated microwells with integrated posts positioned 6mm apart, using an input cell ratio of 75% CM and 25% fibroblasts. Upon compaction, the self-assembled hECT was suspended between the 2 posts, i.e. statically stretched. Morphologically, hECTs had increased cell organization, alignment and density; increased length and alignment of myofibrils and sarcomeres; and increased number and alignment of Z-discs with apparent H-zone, compared to age-matched cell aggregates (Figure 2C). Genetically, the expression of *NPPA* (BNP), *MYL2* (MLC2v) and *MYL7* (MLC2a) were significantly upregulated in hECTs. *MYH7* (β -MHC) expression was significantly upregulated without increased *MYH6* (α -MHC) expression, yielding a significant increased *MYH7/MYH6* expression ratio in hECTs relative to age-matched aggregates. Functionally, the excitation threshold was significantly reduced in hECTs relative to age-matched aggregates but was unaffected by 3 days of electrical stimulation. Conversely, the maximum capture rate was significantly increased in the hECTs relative to age-matched aggregates and was significantly increased after 3 days of electrical stimulation. Pharmacologically, a reduction in Ca^{2+} transient amplitude was demonstrated in response to 500nM verapamil, whereas subsequent treatment with 500nM epinephrine increased the rate of Ca^{2+} transient in hECTs. Treatment with 500nM epinephrine was also demonstrated to increase the AP propagation rate and subsequent treatment with 8.5 μM lidocaine significantly reduced the AP propagation rate in hECTs.

Based on these studies, static stretch platforms promote a variety of morphological improvements (uniform CM distribution; increased alignment of cells, collagen, myofibrils and Z-discs; increased CM size and number; sarcomere development and organization; mitochondria with cristae; increased connexin-43 expression) and signs of genetic

maturation (increased expression of α -MHC, β -MHC, SERCA2a, cardiac α -actin, MLC2v, MLC2a genes). However, these factors are difficult to quantify and compare between the different studies. Fortunately, the functionality and pharmacological responses of the hECTs are more easily compared. In terms of electrophysiology, the statically-stretched hECTs were demonstrated to be capable of excitation-contraction coupling and APD restitution, but were observed to have highly variable APDs and discontinuous AP propagation patterns suggesting statically-stretched hECTs are electrophysiologically immature. Though combining static stretch with a short stint (3 days) of electrical stimulation induced the fastest conduction velocity reported for an hECT (17–33cm/s). The statically-stretched hECTs also generated a maximal contractile stress of 0.50–0.64mN/mm², an order of magnitude lower than the maximal contractile stress reported for hECTs to date. But, the response to various pharmacological compounds was demonstrated to recapitulate some of the adult cardiac tissue responses, e.g. an arrhythmic response to various known pro-arrhythmic compounds and a positive inotropic response to 100nM isoproterenol. However, the EC₅₀ reported for statically-stretched hECTs was 750nM, at least 10-fold higher than reported for adult cardiac muscle.

Relative to static stretch, cyclic stretch was demonstrated to induce some improvements in hECT development. Cyclic stretch induced some further improvements to the morphology of the hECTs, and upregulation of genes and/or proteins associated with maturation and downregulation of “immature” genes/proteins. Cyclically-stretched hECTs were also demonstrated to have faster Ca²⁺ cycling and shorter Ca²⁺ cycle durations, indicating functional improvements.

As a third variation on stretch-conditioning, stepwise-stretch was found to be superior to both static and cyclic stretch with respect to the maximum active and passive force generated, CM and sarcomere alignment, and sarcomere length. Notably, stepwise-stretch induced the generation of 4.4mN/mm² contractile stress, nearly 10-fold higher than achieved by statically-stretched hECTs.

These studies also provide some insight as to the effect of the initial seeding population composition. It was shown that hECTs can be constructed from cardiac bodies as well as from single cells, although this requires that the cardiac bodies have a high degree of CM purity. The addition of fibroblasts was demonstrated to increase the maximal force generated, and to promote uniform cell distribution, synchronous contractions and ECM remodeling. The addition of HUVECs was found to increase CM number, but moreover introduced cord and luminal vessels to the hECT. Supplementation of the medium with ascorbic acid was also found to be advantageous by promoting morphological improvements including intercalated disc development, and increasing the active and passive force generated by the hECTs.

Finally, the utility of stretched hECTs in *in vivo* applications was demonstrated. Implanted statically-stretched hECTs did not invoke a foreign body response, retained sarcomeric organization and cell alignment, and hECTs containing HUVECs anastomosed with the host vasculature. Implanted cyclically-stretch hECTs also retained cell organization, and could

electrically couple with the host myocardium and prevent wall thinning in a rat I/R injury model.

3.2. Electrical stimulation

Suprathreshold electrical stimulation was also used to effectively enhance maturation levels of hPSC-CMs. Nunes *et al* [241] generated hECTs from 35–60% hPSC-CM cell population in a collagen/Matrigel gel seeded around a surgical suture anchored in a 5mm x 1mm x 0.3mm PDMS channel. Upon tissue compaction, the self-assembled hECTs formed a “biowire” along the suture. The hECTs were cultured for 14 days. After 7 days in culture, a subset of hECTs were subjected electrical stimulation (frequency increased to 6Hz incrementally over 7 days). Morphologically, hECTs had increased cell and myofibril alignment, increased numbers of Z-discs, larger CM area and lower proliferation rates, compared with age-matched EBs. Electrically-stimulated hECTs had larger CMs and more rod-shaped CMs than age-matched EBs. Relative to unstimulated hECTs, electrically-stimulated hECTs had increased myofibril convergence, increased organization and alignment of Z-discs, an increased number of H-zones and I-bands per Z-disc, an increased number of mitochondria and desmosomes, increased proximity of mitochondria to the contractile apparatus and nascent intercalated discs (Figure 1E & Figure 2D). Genetically, hECTs had decreased expression of *NPPA* (ANP), *NPPB* (BNP) and *MYH6* (α -MHC), and increased expression of *KCNJ2* (Kir2.1) compared with age-matched EBs. Functionally, the hECTs were demonstrated to beat synchronously and spontaneously. The number of spontaneously beating cells was reduced in hECTs relative to age-matched EBs. Electrical stimulation significantly decreased the excitation threshold and significantly increased the maximum capture rate. Electrical stimulation also increased the conduction velocity of hECTs to ~11.5–18.5cm/s, which was found to be directly related to desmosome abundance. Electrical stimulation also increased I_{Kr} currents, I_{K1} density and cell capacitance. In hECTs, resting membrane potential and APD were decreased, and maximum depolarization rate and AP amplitude were increased relative to age-matched EBs, but were not affected by electrical stimulation. In terms of Ca^{2+} handling, hECTs had increased Ca^{2+} transient amplitude and Ca^{2+} release rate, and decreased time parameters (time to peak amplitude, decay constant and time to baseline), relative to unstimulated hECTs. Pharmacologically, treatment of 5mM caffeine induced an increase in Ca^{2+} transient amplitude in electrically-stimulated hECTs but not unstimulated hECTs. Treatment with 1mM verapamil or 10 μ M nifedipine induced cessation in Ca^{2+} transient in electrically-stimulated hECTs; and subsequent 5mM caffeine treatment induced an increase of cytosolic Ca^{2+} . Treatment of electrically-stimulated hECTs with 2 μ M thapsigargin also induced the cessation of Ca^{2+} transient after a time delay, however subsequent treatment with 5mM caffeine did not evoke a response, which indicated that the SR stores of Ca^{2+} were depleted due to the blocking of SERCA.

Combining static stretch and electrical stimulation in their platform, Hirt *et al* [242] generated hECTs from hiPSC-CMs in a fibrin/Matrigel gel seeded into a 12x3x3mm agarose casting molds in which 2 elastic silicone posts were inserted from above, i.e. the same set-up as Schaaf *et al* [43] but modified to enable electrical stimulation. Upon compaction, the self-assembled hECT was suspended between the 2 posts (static stretch). The hECTs were

cultured for 14 days. After 4 days in culture, a subset of hECTs were subjected to electrical stimulation (2Hz for 1 week and 1.5Hz thereafter); hECTs could be continuously paced for >4 weeks without toxic effects. Morphologically, hECTs had increased cell alignment, an aspect ratio of ~6, more distinct cross-striations and a higher CM-to-ECM ratio, after 10 days of electrical stimulation. Functionally, the contractile force of hECTs increased with electrical stimulation in a time-dependent manner over the first 6–8 days, after which point electrical stimulation had no effect or else a negative effect on force development. Electrical stimulation also induced an increase in fractional shortening, i.e. the difference between the relaxed and contracted length of the hECT.

These studies show that providing the cells with an alignment axis (e.g. suture) promotes morphological (increased cell and myofibril alignment; increased Z-disc and CM numbers) and genetic (decreased expression of “immature” genes and increased expression of Kir2.1 channel) improvements. But also induces electrophysiological improvements (decreased resting membrane potential, decreased APD, increased maximum depolarization rate, increased AP amplitude).

Similarly, electrical conditioning of the hECTs promoted various morphological improvements, including myofibril convergence; more H-zones, I-bands, mitochondria and desmosomes; increased proximity of mitochondria to the contractile apparatus; and nascent intercalated discs. More importantly, electrical conditioning improved the electrical (decreased ET, increased MCR, increased conduction velocity, increased I_{Kr} current, increased I_{K1} density, increased cell capacitance) and Ca^{2+} handling properties (increased Ca^{2+} transient amplitude, increased Ca^{2+} release rate, decreased time to peak amplitude, decreased decay constant, decreased time to baseline, caffeine- and thapsigargin- sensitivity) of the constructs. Notably, electrical conditioning induced a conduction velocity of 11.5–18.5cm/s, among the fastest recorded for hECTs. On the other hand, electrically-stimulated hECTs generated a maximal contractile force of 2.2mN/mm², 2-fold lower than the contractile force generated by stepwise stretch conditioning and 3- to 7-fold lower than the maximal contractile stress generated by an hECT.

3.3 Alignment

Using a platform designed to promote cell alignment, Zhang *et al* [243] investigated the effect of CM purity on the functional maturation of the resultant cardiac tissue. A 3D hECT was generated from 48–90% hESC-CMs in a fibrin/Matrigel gel seeded into a 7x7mm² PDMS mold with staggered hexagonal posts. Upon tissue compaction, a porous tissue was formed. The hECT was cultured for 2 weeks in the presence of ascorbic acid.

Morphologically, the hECTs were uniformly dense, had aligned CMs, and distinct well-developed sarcomeres of adult lengths (2.07–2.11µm). Relative to age-matched 2D cultures, hESC-CMs in hECTs had increased expression of connexin-43 and N-cadherin, but which was localized to the lateral CM borders (Figure 1F). Genetically, *MYH7* (β-MHC) and *MYL2* (MLC2v) expression increased, and *MYH6* (α-MHC) expression decreased, such that the β-MHC/α-MHC and MLC2v/MLC2a expression ratios were significantly increased, relative to the initial hESC-CM seeding population. Furthermore, the expression of *GJA1* (connexin-43) and *SCN5A* (Na_v1.5) increased in hECTs relative to the initial seeding

population of hESC-CMs; and *KCND3* ($K_{v4.3}$), *ATP2A2* (SERCA2) and *CASQ2* (calsequestrin) were all upregulated relative to both hESC-CMs and age-matched 2D cultures. Functionally, the hECTs were demonstrated to have continuous AP propagation with an AP duration in the range of 308–368ms, a maximum capture rate of 2.4–2.8Hz, a maximal conduction velocity of 25.1cm/s, and exhibited both conduction velocity and AP duration restitution (at 1.5–3Hz and 1–3Hz, respectively). The maximum active contractile stress was determined to be $\sim 12\text{mN/mm}^2$; and the hECTs were demonstrated to generate Frank-Starling curves with respect to both active and passive force. In terms of pharmacological sensitivity, a positive chronotropic and inotropic response to isoproterenol was demonstrated for the hECTs with an EC_{50} of 95.1nM determined based on the inotropic response. By varying the CM purity of the initial seeding population, it was demonstrated that APD, maximum capture rate and maximum active force were not affected by CM purity. Conversely, conduction velocity was positively correlated with CM purity; and active force per CM, passive force and contraction time were all negatively correlated with CM purity.

This study once again indicates that platforms designed to promote alignment induce morphological and genetic improvements. However, this porous platform also induced important improvements to the electrical properties (continuous AP propagation; improved APD and MCR; AP duration restitution). Most notably, the platform induced a conduction velocity of 25.1cm/s, on par with the fastest reported for an hECT, as well as the highest maximum contractile stress reported for an hECT (12mN/mm^2). Furthermore, an EC_{50} of 95.1nM for the inotropic response to isoproterenol was demonstrated, near the upper EC_{50} value reported for adult cardiac tissue.

Additionally, it was demonstrated that the CM purity in the hECT can effect various functional parameters. Specifically, conduction velocity increased with CM purity, whereas the active force generated by an individual CM, the passive force and the contraction time decreased with CM purity.

3.4 Decellularized matrix

Using an entirely different approach that built upon the pioneering work of Ott and colleagues [244], Lu *et al* [5] generated hECTs by repopulating whole decellularized mouse hearts with hiPSC-derived multipotential cardiovascular progenitor cells (MCPs). The MCPs were differentiated *in situ* into CMs, smooth muscle cells and endothelial cells (but not fibroblasts) by precisely-timed perfusion of growth factor. The hECTs exhibited myocardium and vessel-like structures. Notably, the atria were not recellularized to the same degree as the ventricles. CMs were identified throughout the recellularized structure and connexin-43 was found to be expressed albeit in a less homogeneous pattern than in native myocardium. After 26 days of culture, the myofibrillar bundles were larger (Figure 2E) and the CM proportion nearly doubled in hECTs relative to age-matched aggregates, which was determined to be the consequence of a 2-fold increase in proliferation rather than decreased apoptosis in hECTs relative to aggregates. Functionally, the hECTs were demonstrated to spontaneously contract after 20 days of perfusion. The hECTs generated a contractile force of $\sim 0.18\text{mN}$ but were found to have a negative force-frequency relationship. While hECTs

were demonstrated to be capable of AP propagation, they had an irregular AP shape. Similarly, the hECTs constructs were observed to have functional but asynchronous Ca^{2+} transient. Additionally, anatomical blocks were occasionally observed that prevented continuous AP propagation, but which could be synchronized by means of electrical stimulation. Genetically, the hECT expression of *MYH6* (α -MHC), *TNNT2* (cardiac troponin-T), *GJA1* (connexin-43) and *NKX2-5*, a transcription factor for cardiac mesoderm specification [245], was demonstrated to be upregulated relative to age-matched aggregates, moreover the expression levels in the hECTs were in the range expressed in fetal and adult tissue. *MYH7* (β -MHC) expression was significantly upregulated in hECTs relative to age-matched aggregates but also significantly less than found in either fetal or adult human tissue. Pharmacologically, hECTs were demonstrated to have a positive inotropic response to increasing extracellular Ca^{2+} (1.25 to 5mM) and a positive chronotropic response to increasing isoproterenol concentrations with an EC_{50} of 10–100nM. Treatment with 1 μ M isoproterenol increased Ca^{2+} transient frequency and decreased Ca^{2+} transient amplitude. Treatment with 1 μ M E-4031 induced a significantly decreased Ca^{2+} transient amplitude as well as an arrhythmic pulsing pattern, i.e. an alternating strong and weak pulse (pulsus alternans).

4. Advantages of maturation in 3D and challenges ahead

Compared to the current alternatives, 2D cell culture and *ex vivo* myocardial slices, hECTs culture enables an approximation of native tissue and longer culture periods, respectively. Moreover, hPSC-CMs in hECTs have achieved the highest degree of maturation to date in terms of functional parameters. 2D culture also requires a significantly longer culture times (180 + days) to achieve a higher degrees of maturation compared to hECT culture that usually takes 14–28 days. Despite these achievements, several structural and functional characteristics are still not robustly achieved, namely generation of T-tubules, M-lines and a robust positive force-frequency relationship in hECTs.

Furthermore, hECTs remain a simplified model that lacks many components of a working heart, such as humoral, metabolic, and neurogenic factors that affect cardiac function and interventional responses. This limitation becomes apparent in reports of isolated native heart tissues being insensitive to known arrhythmogens [246]. Regardless, the continued development of drug testing platforms based on human cell sources is strongly supported by species-specific cardiac drug responses [247, 248].

Looking towards personalized medicine, it is theoretically possible to create hECTs created from patient-specific iPSCs [249–251]. In fact, functional CMs were generated from the hiPSCs of advanced heart failure patients [252]. Furthermore, the stem cell field has progressed such that hiPSCs can now be generated from a wide variety of easily accessible somatic cell sources—keratinocytes from skin or plucked hair [253], peripheral blood [254], mesenchymal cells in fat [255], dental pulp [256], and oral mucosa [257]—with minimal burden to the patient. Patient-specific iPSCs in combination with hECT technology could be used to generate patient-specific tissues for personalized medicine approaches thereby providing personalized *in vitro* drug screening, therapeutic strategies and enabling the identification of drug sensitivities. Similarly, patient-specific tissues could help to advance

our understanding of the pathophysiology of monogenic and complex diseases, particularly in the case of rare diseases [192].

Clinical application and the generation of thicker hECTs will require the challenge of vascularization be addressed. An intrinsic vasculature is important for the construction of larger hECTs, to permit nutrient and oxygen exchange with cells beyond the reach of diffusion and for successful implantation, to enable the implanted tissue to be perfused by the host vasculature. The addition of endothelial cells and MSCs has been demonstrated to promote hECT vascularization and enable perfusion by host vasculature upon implantation [32], and there is a consensus in the field now that co-culture or tri-culture is required for both enhanced cardiac maturation and to enable survival *in vivo*. Another method might entail the use cardiovascular progenitor cells capable of differentiating into the necessary cardiac cell types *in situ* [20, 258, 259]. Alternatively, the porous cardiac patch designed by Zhang *et al* [243] improved nutrient and oxygen transport, and could be a means of developing thicker hECTs. Clinical application will also require that undefined matrix components be replaced. To this end, Kensah *et al* [34] demonstrated that Matrigel could be substituted with a defined animal-free matrix (collagen and hyaluronic acid) without significantly affecting the resultant hECT.

Of course the main obstacle to realizing the potential of hECTs is that currently they are still a poor approximation of human adult myocardium. A variety of different strategies have been investigated to advance the development of hPSC-CMs towards the adult phenotype. Further levels of maturation could additionally be achieved by combining multiple stimuli such as electrical stimulation [13, 30, 31], mechanical conditioning [13, 32–34], substrate stiffness and/or topology [30, 35], and chemical manipulation [29, 34], e.g. ascorbic acid [34], growth factors or hormones—triiodothyronine [260], neuregulin-1 β [159] or insulin-like growth factor-1 [261]. Both topographical cues and ECM patterning have been demonstrated to improve the maturity of cardiac tissues, which due to a recent innovation by Sun *et al* [262] can now be used in combination. Timed regulation of gene expression in hECT cultures by means of adenoviral-mediated gene transfer has been suggested as a means of directed hPSC-CM maturation and its feasibility was demonstrated [193]. The non-myocyte cell fraction also contributes significantly to maturation [32, 260, 263–265]. Specifically, ion channel development and electrophysiological maturation of early hESC-CM cultures was demonstrated to be dependent upon the presence of non-myocytes [140].

Much like the challenge of maturing hPSC-CMs, the classification of CMs as mature is not trivial. A controversy surrounds the classification of cardiac cells derived from hPSCs or direct reprogramming on the basis of molecular, structural, and physiological markers [266, 267]. First, there is no consensus as to which markers should be used to identify CMs. Some of the frequently used cardiac markers are not entirely unique to CMs, e.g. cardiac troponin-T is expressed by smooth muscle cells [268, 269]. Second, a major challenge in tracking CM maturity is that markers in frequent use are highly plastic in nature, which makes it difficult to identify mature CMs. Genes expressed during the early stages of development are also reactivated during a variety of pathophysiologic conditions, including hypoxia, ischemia, hypertrophy, atrophy, diabetes, and hypothyroidism. The failing adult CM downregulates adult gene transcripts and reactivates the fetal program at the structural, hormonal, and

metabolic level [268, 269]. In heart failure and pathological hypertrophy, there is a downregulation of genes used to identify the adult CM phenotype, e.g. *MYH6* (α -MHC), *ATP2A2* (SERCA2a), various ion channels and *GLUT4* (glucose transporter 4) [94, 270–274]; and an upregulation of genes expressed in the fetal heart—*NPPA* (ANP), *NPPB* (BNP), *ACT1A* (α -skeletal actin) and *GLUT1* (glucose transporter 1) [270, 271, 273]. This reversion to a fetal phenotype extends to the functional maturity assessments in that a flat or negative force-frequency curve is characteristic of both fetal/neonatal hearts [184] and failing adult hearts [275, 276]. As a result, it is challenging to distinguish between a fetal stage of development or a reversion to the fetal phenotype as consequence of disease.

Thirdly, certain markers can provide a false assessment of maturity if considered in isolation. For example, while most hPSC-CMs express MLC2a and not MLC2v at early stages of development and robustly express MLC2v at later stages, it has been observed that MLC2a continues to be overexpressed in the late stage hPSC-CMs. Moreover, hPSC-CMs robustly expressing MLC2v have been demonstrated to also express the slow skeletal isoform of troponin-I associated with fetal heart tissue [44]. It has therefore been suggested that the co-localization of cardiac troponin-I with MLC2v may provide a more accurate classification of mature ventricular CMs [44]. Similarly, contractile force is frequently measured under strong field stimulation [32, 264, 277]. This method assesses the maximum contractile force generated by all the CMs that have been excited simultaneously, but does not consider the degree of coupling that exists within the tissue. Point stimulation at near-threshold pacing could be used to initiate AP propagation and thereby induce contraction in a more physiological manner. Fourthly, a number of the standard markers used are highly dependent upon the measurement conditions. Excitation threshold is highly dependent on the orientation of the cells relative to the electrodes, the geometry of the electrode and bath and the presence of structural anomalies in the tissue [278]. The amplitude of the tissue contraction is also highly dependent on the stiffness of the tissue construct, local cell orientation and tissue geometry, factors which themselves are influenced by culture time and conditions. The culture device may also influence measurements, as seen by the near equivalent active force (0.12mN/mm^2 and 0.13mN/mm^2) reported by Schaaf *et al* [43] and Turnbull *et al* [193] using similar PDMS devices despite differences in scaffold, cell purity and culture conditions; and the observation that the force calculated from post-deflection were 4-fold lower than measured for the same hECTs in the physiological muscle bath.

Despite these challenges, our understanding of the similarities and differences between engineered and native human myocardium, and how that transition might be induced continues to grow; and with it the potential for hECTs to be developed for preclinical therapeutic screening and regenerative medicine. As the field of cardiac tissue engineering continues to advance, the promise of a reliable *in vitro* surrogate for human myocardium nears reality.

5. Summary

The multidisciplinary field of cardiac tissue engineering continues to progress and to contribute to our ever growing understanding of the definition of CM maturity, the methods by which maturity can be assessed and the ways in which hPSC-CMs can be matured *in*

vitro. To date, ECTs have demonstrated the greatest potential to promote the maturation of CMs, and while some functional measures have achieved near adult levels, many others are far from achieving this benchmark. A surrogate for adult cardiac tissue has yet to be achieved but the field is swiftly finding means of advancing individual phenotypic markers to the adult range. Each hECT generated has demonstrated a strength in promoting the advancement of a subset of maturation markers, suggesting the need for a combinatorial approach incorporating the best of each design. A lack of standardization in the methods used to assess maturity and a lack of a consensus as to what constitutes the mature CM phenotype are hindrances to advancement but are not insurmountable. Moreover, individual studies have demonstrated that hECTs can be vascularized and perfused by host vasculature upon implantation, can functionally integrate with host myocardium *in vivo*, and are sensitive to cardiotoxic and/or proarrhythmic drugs. This body of data is not vast but suggests that the current state of enthusiasm and rapid discovery are warranted and a shift from the bench to the clinic may be on the horizon.

Acknowledgments

This work is funded by the Heart and Stoke Foundation GIA T6946, the Canadian Institutes of Health Research (CIHR) Operating Grant (MOP-126027), NSERC Discovery Grant (RGPIN 326982-10), and National Institutes of Health grant 2R01 HL076485. M.R. is supported by Canada Research Chair (Tier 2) and Steacie Fellowship.

References

1. Alwan A, Armstrong T, Bettcher D, Branca F, Chisholm D, Ezzati M, Garfield R, MacLean D, Mathers C, Mendis S, Poznyak V, Riley L, Tang KC, Wild C. Global status report on noncommunicable diseases. 2011; 2010
2. Moran AE, Forouzanfar MH, Roth GA, Mensah GA, Ezzati M, Murray CJ, Naghavi M. Temporal trends in ischemic heart disease mortality in 21 world regions, 1980 to 2010: the Global Burden of Disease 2010 study. *Circulation*. 2014; 129:1483–1492. [PubMed: 24573352]
3. Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. *PLoS Med*. 2006; 3:e442. [PubMed: 17132052]
4. Rosamond W, Flegal K, Furie K, Go A, Greenlund K, Haase N, Hailpern SM, Ho M, Howard V, Kissela B, Kittner S, Lloyd-Jones D, McDermott M, Meigs J, Moy C, Nichol G, O'Donnell C, Roger V, Sorlie P, Steinberger J, Thom T, Wilson M, Hong Y. American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics--2008 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. *Circulation*. 2008; 117:e25–146. [PubMed: 18086926]
5. Lu TY, Lin B, Kim J, Sullivan M, Tobita K, Salama G, Yang L. Repopulation of decellularized mouse heart with human induced pluripotent stem cell-derived cardiovascular progenitor cells. *Nat Commun*. 2013; 4:2307. [PubMed: 23942048]
6. Minotti, G., editor. *Cardiotoxicity of Non-Cardiovascular Drugs*. John Wiley & Sons, Ltd; Chichester, UK: 2010. Front Matter.
7. Piccini JP, Whellan DJ, Berridge BR, Finkle JK, Pettit SD, Stockbridge N, Valentin JP, Vargas HM, Krucoff MW. CSRC/HESI Writing Group. Current challenges in the evaluation of cardiac safety during drug development: translational medicine meets the Critical Path Initiative. *Am Heart J*. 2009; 158:317–326. [PubMed: 19699852]
8. Eschenhagen, T.; Wakatsuki, T.; Elson, EL. A new method to measure isometric force of contraction in embryonic cardiac myocytes. Report No. 95-17 on the Second International Conference on Cellular Engineering; LaJolla. August 19–22 (1995);
9. Hirt MN, Hansen A, Eschenhagen T. Cardiac tissue engineering: state of the art. *Circ Res*. 2014; 114:354–367. [PubMed: 24436431]

10. Sawa Y, Miyagawa S, Sakaguchi T, Fujita T, Matsuyama A, Saito A, Shimizu T, Okano T. Tissue engineered myoblast sheets improved cardiac function sufficiently to discontinue LVAS in a patient with DCM: report of a case. *Surg Today*. 2012; 42:181–184. [PubMed: 22200756]
11. Zvibel I, Smets F, Soriano H, Anokis: roadblock to cell transplantation? *Cell Transplant*. 2002; 11:621–630. [PubMed: 12518889]
12. Robey TE, Saiget MK, Reinecke H, Murry CE. Systems approaches to preventing transplanted cell death in cardiac repair. *J Mol Cell Cardiol*. 2008; 45:567–581. [PubMed: 18466917]
13. Boudou T, Legant WR, Mu A, Borochin MA, Thavandiran N, Radisic M, Zandstra PW, Epstein JA, Margulies KB, Chen CS. A microfabricated platform to measure and manipulate the mechanics of engineered cardiac microtissues. *Tissue Eng Part A*. 2012; 18:910–919. [PubMed: 22092279]
14. Dengler J, Song H, Thavandiran N, Masse S, Wood GA, Nanthakumar K, Zandstra PW, Radisic M. Engineered heart tissue enables study of residual undifferentiated embryonic stem cell activity in a cardiac environment. *Biotechnol Bioeng*. 2011; 108:704–719. [PubMed: 21246514]
15. Grosberg A, Alford PW, McCain ML, Parker KK. Ensembles of engineered cardiac tissues for physiological and pharmacological study: heart on a chip. *Lab Chip*. 2011; 11:4165–4173. [PubMed: 22072288]
16. Hansen A, Eder A, Bonstrup M, Flato M, Mewe M, Schaaf S, Aksehirlioglu B, Schwoerer AP, Uebeler J, Eschenhagen T. Development of a drug screening platform based on engineered heart tissue. *Circ Res*. 2010; 107:35–44. [PubMed: 20448218]
17. Vandenburgh H, Shansky J, Benesch-Lee F, Skelly K, Spinazzola JM, Saponjian Y, Tseng BS. Automated drug screening with contractile muscle tissue engineered from dystrophic myoblasts. *FASEB J*. 2009; 23:3325–3334. [PubMed: 19487307]
18. Song H, Yoon C, Kattman SJ, Dengler J, Masse S, Thavaratnam T, Gewarges M, Nanthakumar K, Rubart M, Keller GM, Radisic M, Zandstra PW. Interrogating functional integration between injected pluripotent stem cell-derived cells and surrogate cardiac tissue. *Proc Natl Acad Sci U S A*. 2010; 107:3329–3334. [PubMed: 19846783]
19. Kraushaar U, Meyer T, Hess D, Gepstein L, Mummery CL, Braam SR, Guenther E. Cardiac safety pharmacology: from human ether-a-gogo related gene channel block towards induced pluripotent stem cell based disease models. *Expert Opin Drug Saf*. 2012; 11:285–298. [PubMed: 22103746]
20. Yang L, Soonpaa MH, Adler ED, Roepke TK, Kattman SJ, Kennedy M, Henckaerts E, Bonham K, Abbott GW, Linden RM, Field LJ, Keller GM. Human cardiovascular progenitor cells develop from a KDR+ embryonic-stem-cell-derived population. *Nature*. 2008; 453:524–528. [PubMed: 18432194]
21. Kattman SJ, Witty AD, Gagliardi M, Dubois NC, Niapour M, Hotta A, Ellis J, Keller G. Stage-specific optimization of activin/nodal and BMP signaling promotes cardiac differentiation of mouse and human pluripotent stem cell lines. *Cell Stem Cell*. 2011; 8:228–240. [PubMed: 21295278]
22. Lian X, Zhang J, Azarin SM, Zhu K, Hazeltine LB, Bao X, Hsiao C, Kamp TJ, Palecek SP. Directed cardiomyocyte differentiation from human pluripotent stem cells by modulating Wnt/beta-catenin signaling under fully defined conditions. *Nat Protoc*. 2013; 8:162–175. [PubMed: 23257984]
23. BurrIDGE PW, Thompson S, Millrod MA, Weinberg S, Yuan X, Peters A, Mahairaki V, Koliatsos VE, Tung L, Zambidis ET. A universal system for highly efficient cardiac differentiation of human induced pluripotent stem cells that eliminates interline variability. *PLoS One*. 2011; 6:e18293. [PubMed: 21494607]
24. BurrIDGE PW, Matsa E, Shukla P, Lin ZC, Churko JM, Ebert AD, Lan F, Diecke S, Huber B, Mordwinkin NM, Plews JR, Abilez OJ, Cui B, Gold JD, Wu JC. Chemically defined generation of human cardiomyocytes. *Nat Methods*. 2014; 11:855–860. [PubMed: 24930130]
25. Laflamme MA, Chen KY, Naumova AV, Muskheili V, Fugate JA, Dupras SK, Reinecke H, Xu C, Hassanipour M, Police S, O'Sullivan C, Collins L, Chen Y, Minami E, Gill EA, Ueno S, Yuan C, Gold J, Murry CE. Cardiomyocytes derived from human embryonic stem cells in pro-survival factors enhance function of infarcted rat hearts. *Nat Biotechnol*. 2007; 25:1015–1024. [PubMed: 17721512]

26. Chen HS, Kim C, Mercola M. Electrophysiological challenges of cell-based myocardial repair. *Circulation*. 2009; 120:2496–2508. [PubMed: 20008740]
27. Blin G, Nury D, Stefanovic S, Neri T, Guillevic O, Brinon B, Bellamy V, Rucker-Martin C, Barbry P, Bel A, Bruneval P, Cowan C, Pouly J, Mitalipov S, Gouadon E, Binder P, Hagege A, Desnos M, Renaud JF, Menasche P, Puceat M. A purified population of multipotent cardiovascular progenitors derived from primate pluripotent stem cells engrafts in postmyocardial infarcted nonhuman primates. *J Clin Invest*. 2010; 120:1125–1139. [PubMed: 20335662]
28. Adler ED, Chen VC, Bystrup A, Kaplan AD, Giovannone S, Briley-Saebo K, Young W, Kattman S, Mani V, Laflamme M, Zhu WZ, Fayad Z, Keller G. The cardiomyocyte lineage is critical for optimization of stem cell therapy in a mouse model of myocardial infarction. *FASEB J*. 2010; 24:1073–1081. [PubMed: 19940262]
29. Pillekamp F, Hausteim M, Khalil M, Emmelheinz M, Nazzari R, Adelmann R, Nguemo F, Rubenchyk O, Pfannkuche K, Matzkies M, Reppel M, Bloch W, Brockmeier K, Hescheler J. Contractile properties of early human embryonic stem cell-derived cardiomyocytes: beta-adrenergic stimulation induces positive chronotropy and lusitropy but not inotropy. *Stem Cells Dev*. 2012; 21:2111–2121. [PubMed: 22268955]
30. Heidi Au HT, Cui B, Chu ZE, Veres T, Radisic M. Cell culture chips for simultaneous application of topographical and electrical cues enhance phenotype of cardiomyocytes. *Lab Chip*. 2009; 9:564–575. [PubMed: 19190792]
31. Radisic M, Park H, Shing H, Consi T, Schoen FJ, Langer R, Freed LE, Vunjak-Novakovic G. Functional assembly of engineered myocardium by electrical stimulation of cardiac myocytes cultured on scaffolds. *Proc Natl Acad Sci U S A*. 2004; 101:18129–18134. [PubMed: 15604141]
32. Tulloch NL, Muskheli V, Razumova MV, Korte FS, Regnier M, Hauch KD, Pabon L, Reinecke H, Murry CE. Growth of engineered human myocardium with mechanical loading and vascular coculture. *Circ Res*. 2011; 109:47–59. [PubMed: 21597009]
33. Akhyari P, Fedak PW, Weisel RD, Lee TY, Verma S, Mickle DA, Li RK. Mechanical stretch regimen enhances the formation of bioengineered autologous cardiac muscle grafts. *Circulation*. 2002; 106:1137–42. [PubMed: 12354723]
34. Kensah G, Gruh I, Viering J, Schumann H, Dahlmann J, Meyer H, Skvorc D, Bar A, Akhyari P, Heisterkamp A, Haverich A, Martin U. A novel miniaturized multimodal bioreactor for continuous in situ assessment of bioartificial cardiac tissue during stimulation and maturation. *Tissue Eng Part C Methods*. 2011; 17:463–473. [PubMed: 21142417]
35. Bhana B, Iyer RK, Chen WL, Zhao R, Sider KL, Likhitanichkul M, Simmons CA, Radisic M. Influence of substrate stiffness on the phenotype of heart cells. *Biotechnol Bioeng*. 2010; 105:1148–1160. [PubMed: 20014437]
36. Synnergren J, Ameen C, Jansson A, Sartipy P. Global transcriptional profiling reveals similarities and differences between human stem cell-derived cardiomyocyte clusters and heart tissue. *Physiol Genomics*. 2012; 44:245–258. [PubMed: 22166955]
37. 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 AB, Passier R, Tertoolen L. Differentiation of human embryonic stem cells to cardiomyocytes: role of coculture with visceral endoderm-like cells. *Circulation*. 2003; 107:2733–2740. [PubMed: 12742992]
38. Robertson C, Tran DD, George SC. Concise review: maturation phases of human pluripotent stem cell-derived cardiomyocytes. *Stem Cells*. 2013; 31:829–837. [PubMed: 23355363]
39. Mummery CL, Zhang J, Ng ES, Elliott DA, Elefanty AG, Kamp TJ. Differentiation of human embryonic stem cells and induced pluripotent stem cells to cardiomyocytes: a methods overview. *Circ Res*. 2012; 111:344–358. [PubMed: 22821908]
40. O'Hara T, Virag L, Varro A, Rudy Y. Simulation of the undiseased human cardiac ventricular action potential: model formulation and experimental validation. *PLoS Comput Biol*. 2011; 7:e1002061. [PubMed: 21637795]
41. Poon E, Kong CW, Li RA. Human pluripotent stem cell-based approaches for myocardial repair: from the electrophysiological perspective. *Mol Pharm*. 2011; 8:1495–1504. [PubMed: 21879736]

42. Itzhaki I, Schiller J, Beyar R, Satin J, Gepstein L. Calcium handling in embryonic stem cell-derived cardiac myocytes: of mice and men. *Ann N Y Acad Sci.* 2006; 1080:207–215. [PubMed: 17132785]
43. Schaaf S, Shibamiya A, Mewe M, Eder A, Stohr A, Hirt MN, Rau T, Zimmermann WH, Conradi L, Eschenhagen T, Hansen A. Human engineered heart tissue as a versatile tool in basic research and preclinical toxicology. *PLoS One.* 2011; 6:e26397. [PubMed: 22028871]
44. Bedada FB, Chan SS, Metzger SK, Zhang L, Zhang J, Garry DJ, Kamp TJ, Kyba M, Metzger JM. Acquisition of a quantitative, stoichiometrically conserved ratiometric marker of maturation status in stem cell-derived cardiac myocytes. *Stem Cell Reports.* 2014; 3:594–605. [PubMed: 25358788]
45. Gerdes AM, Kellerman SE, Moore JA, Muffly KE, Clark LC, Reaves PY, Malec KB, McKeown PP, Schocken DD. Structural remodeling of cardiac myocytes in patients with ischemic cardiomyopathy. *Circulation.* 1992; 86:426–430. [PubMed: 1638711]
46. Mailliet M, van Berlo JH, Molkentin JD. Molecular basis of physiological heart growth: fundamental concepts and new players. *Nat Rev Mol Cell Biol.* 2013; 14:38–48. [PubMed: 23258295]
47. Yang X, Pabon L, Murry CE. Engineering adolescence: maturation of human pluripotent stem cell-derived cardiomyocytes. *Circ Res.* 2014; 114:511–523. [PubMed: 24481842]
48. Soonpaa MH, Field LJ. Survey of studies examining mammalian cardiomyocyte DNA synthesis. *Circ Res.* 1998; 83:15–26. [PubMed: 9670914]
49. Colomer JM, Terasawa M, Means AR. Targeted expression of calmodulin increases ventricular cardiomyocyte proliferation and deoxyribonucleic acid synthesis during mouse development. *Endocrinology.* 2004; 145:1356–1366. [PubMed: 14670993]
50. Chaudhry HW, Dashoush NH, Tang H, Zhang L, Wang X, Wu EX, Wolgemuth DJ. Cyclin A2 mediates cardiomyocyte mitosis in the postmitotic myocardium. *J Biol Chem.* 2004; 279:35858–35866. [PubMed: 15159393]
51. Bergmann O, Bhardwaj RD, Bernard S, Zdunek S, Barnabe-Heider F, Walsh S, Zupicich J, Alkass K, Buchholz BA, Druid H, Jovinge S, Frisen J. Evidence for cardiomyocyte renewal in humans. *Science.* 2009; 324:98–102. [PubMed: 19342590]
52. Li RK, Mickle DA, Weisel RD, Carson S, Omar SA, Tumati LC, Wilson GJ, Williams WG. Human pediatric and adult ventricular cardiomyocytes in culture: assessment of phenotypic changes with passaging. *Cardiovasc Res.* 1996; 32:362–373. [PubMed: 8796124]
53. Ribeiro MC, Tertoolena LG, Guadix JA, Bellina M, Kosmidisa G, D'Aniello C, Monshouer-Klootsa J, Goumans MJ, Wang YL, Feinberg AW, Mummery CL, Passiera R. Functional maturation of human pluripotent stem cell derived cardiomyocytes *in vitro* – Correlation between contraction force and electrophysiology. *Biomaterials.* 2015; 51:138–150. [PubMed: 25771005]
54. Spach MS, Heidlage JF, Barr RC, Dolber PC. Cell size and communication: role in structural and electrical development and remodeling of the heart. *Heart Rhythm.* 2004; 1:500–515. [PubMed: 15851207]
55. Dorn GW 2nd, Robbins J, Sugden PH. Phenotyping hypertrophy: eschew obfuscation. *Circ Res.* 2003; 92:1171–1175. [PubMed: 12805233]
56. Carreno JE, Apablaza F, Ocaranza MP, Jalil JE. Cardiac hypertrophy: molecular and cellular events. *Rev Esp Cardiol.* 2006; 59:473–486. [PubMed: 16750145]
57. Black LD 3rd, Meyers JD, Weinbaum JS, Shvelidze YA, Tranquillo RT. Cell-induced alignment augments twitch force in fibrin gel-based engineered myocardium via gap junction modification. *Tissue Eng Part A.* 2009; 15:3099–3108. [PubMed: 19338433]
58. Chung CY, Bien H, Entcheva E. The role of cardiac tissue alignment in modulating electrical function. *J Cardiovasc Electrophysiol.* 2007; 18:1323–1329. [PubMed: 17916158]
59. Rubin, R.; Strayer, DS., editors. *Rubin's pathology: clinicopathologic foundations of medicine.* Wolters Kluwer Health/Lippincott Williams & Wilkins; Philadelphia: 2012.
60. Lundy SD, Zhu WZ, Regnier M, Laflamme MA. Structural and functional maturation of cardiomyocytes derived from human pluripotent stem cells. *Stem Cells Dev.* 2013; 22:1991–2002. [PubMed: 23461462]
61. Yang L, Soonpaa MH, Adler ED, Roepke TK, Kattman SJ, Kennedy M, Henckaerts E, Bonham K, Abbott GW, Linden RM, Field LJ, Keller GM. Human cardiovascular progenitor cells develop

- from a KDR+ embryonic-stem-cell-derived population. *Nature*. 2008; 453:524–528. [PubMed: 18432194]
62. Xu XQ, Graichen R, Soo SY, Balakrishnan T, Rahmat SN, Sieh S, Tham SC, Freund C, Moore J, Mummery C, Colman A, Zweigerdt R, Davidson BP. Chemically defined medium supporting cardiomyocyte differentiation of human embryonic stem cells. *Differentiation*. 2008; 76:958–970. [PubMed: 18557764]
 63. Feinberg AW, Alford PW, Jin H, Ripplinger CM, Werdich AA, Sheehy SP, Grosberg A, Parker KK. Controlling the contractile strength of engineered cardiac muscle by hierarchical tissue architecture. *Biomaterials*. 2012; 33:5732–5741. [PubMed: 22594976]
 64. Kamakura T, Makiyama T, Sasaki K, Yoshida Y, Wuriyanghai Y, Chen J, Hattori T, Ohno S, Kita T, Horie M, Yamanaka S, Kimura T. Ultrastructural maturation of human-induced pluripotent stem cell-derived cardiomyocytes in a long-term culture. *Circ J*. 2013; 77:1307–1314. [PubMed: 23400258]
 65. Snir M, Kehat I, Gepstein A, Coleman R, Itskovitz-Eldor J, Livne E, Gepstein L. Assessment of the ultrastructural and proliferative properties of human embryonic stem cell-derived cardiomyocytes. *Am J Physiol Heart Circ Physiol*. 2003; 285:H2355–63. [PubMed: 14613910]
 66. Satin J, Itzhaki I, Rapoport S, Schroder EA, Izu L, Arbel G, Beyar R, Balke CW, Schiller J, Gepstein L. Calcium handling in human embryonic stem cell-derived cardiomyocytes. *Stem Cells*. 2008; 26:1961–1972. [PubMed: 18483424]
 67. Haddock PS, Coetzee WA, Cho E, Porter L, Katoh H, Bers DM, Jafri MS, Artman M. Subcellular [Ca²⁺]_i gradients during excitation-contraction coupling in newborn rabbit ventricular myocytes. *Circ Res*. 1999; 85:415–427. [PubMed: 10473671]
 68. Seki S, Nagashima M, Yamada Y, Tsutsuura M, Kobayashi T, Namiki A, Tohse N. Fetal and postnatal development of Ca²⁺ transients and Ca²⁺ sparks in rat cardiomyocytes. *Cardiovasc Res*. 2003; 58:535–548. [PubMed: 12798426]
 69. Lieu DK, Liu J, Siu CW, McNerney GP, Tse HF, Abu-Khalil A, Huser T, Li RA. Absence of transverse tubules contributes to non-uniform Ca(2+) wavefronts in mouse and human embryonic stem cell-derived cardiomyocytes. *Stem Cells Dev*. 2009; 18:1493–1500. [PubMed: 19290776]
 70. Vreeker A, van Stuijvenberg L, Hund TJ, Mohler PJ, Nikkels PG, van Veen TA. Assembly of the cardiac intercalated disk during pre- and postnatal development of the human heart. *PLoS One*. 2014; 9:e94722. [PubMed: 24733085]
 71. Noorman M, van der Heyden MA, van Veen TA, Cox MG, Hauer RN, de Bakker JM, van Rijen HV. Cardiac cell-cell junctions in health and disease: Electrical versus mechanical coupling. *J Mol Cell Cardiol*. 2009; 47:23–31. [PubMed: 19344726]
 72. Jansen JA, van Veen TA, de Bakker JM, van Rijen HV. Cardiac connexins and impulse propagation. *J Mol Cell Cardiol*. 2010; 48:76–82. [PubMed: 19729017]
 73. Zwi L, Caspi O, Arbel G, Huber I, Gepstein A, Park IH, Gepstein L. Cardiomyocyte differentiation of human induced pluripotent stem cells. *Circulation*. 2009; 120:1513–1523. [PubMed: 19786631]
 74. Peters NS, Severs NJ, Rothery SM, Lincoln C, Yacoub MH, Green CR. Spatiotemporal relation between gap junctions and fascia adherens junctions during postnatal development of human ventricular myocardium. *Circulation*. 1994; 90:713–725. [PubMed: 8044940]
 75. Angst BD, Khan LU, Severs NJ, Whitely K, Rothery S, Thompson RP, Magee AI, Gourdie RG. Dissociated spatial patterning of gap junctions and cell adhesion junctions during postnatal differentiation of ventricular myocardium. *Circ Res*. 1997; 80:88–94. [PubMed: 8978327]
 76. Bartelds B, Knoester H, Smid GB, Takens J, Visser GH, Penninga L, van der Leij FR, Beaufort-Krol GC, Zijlstra WG, Heymans HS, Kuipers JR. Perinatal changes in myocardial metabolism in lambs. *Circulation*. 2000; 102:926–931. [PubMed: 10952964]
 77. Porter GA Jr, Hom J, Hoffman D, Quintanilla R, de Mesy Bentley K, Sheu SS. Bioenergetics, mitochondria, and cardiac myocyte differentiation. *Prog Pediatr Cardiol*. 2011; 31:75–81. [PubMed: 21603067]
 78. Navaratnam, V., editor. *Heart muscle : ultrastructural studies*. Cambridge University Press; New York: 1987.
 79. Pinnell J, Turner S, Howell S. Cardiac muscle physiology, *Continuing Education in Anaesthesia, Critical Care and Pain*. 2007; 7:85–88.

80. Ong SB, Hall AR, Hausenloy DJ. Mitochondrial dynamics in cardiovascular health and disease. *Antioxid Redox Signal*. 2013; 19:400–414. [PubMed: 22793879]
81. Gorza L, Mercadier JJ, Schwartz K, Thornell LE, Sartore S, Schiaffino S. Myosin types in the human heart. An immunofluorescence study of normal and hypertrophied atrial and ventricular myocardium. *Circ Res*. 1984; 54:694–702. [PubMed: 6234108]
82. Saggin L, Gorza L, Ausoni S, Schiaffino S. Troponin I switching in the developing heart. *J Biol Chem*. 1989; 264:16299–16302. [PubMed: 2777792]
83. Bodi I, Mikala G, Koch SE, Akhter SA, Schwartz A. The L-type calcium channel in the heart: the beat goes on. *J Clin Invest*. 2005; 115:3306–3317. [PubMed: 16322774]
84. Weiss S, Oz S, Benmocha A, Dascal N. Regulation of cardiac L-type Ca^{2+} channel $\text{Ca}_v1.2$ via the beta-adrenergic-cAMP-protein kinase A pathway: old dogmas, advances, and new uncertainties. *Circ Res*. 2013; 113:617–631. [PubMed: 23948586]
85. Sartiani L, Bettiol E, Stillitano F, Mugelli A, Cerbai E, Jaconi ME. Developmental changes in cardiomyocytes differentiated from human embryonic stem cells: a molecular and electrophysiological approach. *Stem Cells*. 2007; 25:1136–1144. [PubMed: 17255522]
86. Mercier A, Clement R, Harnois T, Bourmeyster N, Faivre JF, Findlay I, Chahine M, Bois P, Chatelier A. The beta1-subunit of $\text{Na}^+\text{v}1.5$ cardiac sodium channel is required for a dominant negative effect through alpha-alpha interaction. *PLoS One*. 2012; 7:e48690. [PubMed: 23133651]
87. Giudicessi JR, Ye D, Kritzer CJ, Nesterenko VV, Tester DJ, Antzelevitch C, Ackerman MJ. Novel mutations in the KCND3-encoded $\text{Kv}4.3$ K^+ channel associated with autopsy-negative sudden unexplained death. *Hum Mutat*. 2012; 33:989–997. [PubMed: 22457051]
88. Blazewski A, Zhu R, Hunter DW, Weinberg SH, Zambidis ET, Tung L. Cardiomyocytes derived from human induced pluripotent stem cells as models for normal and diseased cardiac electrophysiology and contractility. *Prog Biophys Mol Biol*. 2012; 110:166–177. [PubMed: 22971665]
89. Vega AL, Tester DJ, Ackerman MJ, Makielski JC. Protein kinase A-dependent biophysical phenotype for V227F-KCNJ2 mutation in catecholaminergic polymorphic ventricular tachycardia. *Circ Arrhythm Electrophysiol*. 2009; 2:540–547. [PubMed: 19843922]
90. Silva J, Rudy Y. Mechanism of pacemaking in I(K1)-downregulated myocytes. *Circ Res*. 2003; 92:261–263. [PubMed: 12595336]
91. Gaborit N, Le Bouter S, Szuts V, Varro A, Escande D, Nattel S, Demolombe S. Regional and tissue specific transcript signatures of ion channel genes in the non-diseased human heart. *J Physiol*. 2007; 582:675–693. [PubMed: 17478540]
92. Lipskaia L, Chemaly ER, Hadri L, Lompre AM, Hajjar RJ. Sarcoplasmic reticulum Ca^{2+} -ATPase as a therapeutic target for heart failure. *Expert Opin Biol Ther*. 2010; 10:29–41. [PubMed: 20078230]
93. Dirksen WP, Lacombe VA, Chi M, Kalyanasundaram A, Viatchenko-Karpinski S, Terentyev D, Zhou Z, Vedamoorthy S, Li N, Chiamvimonvat N, Carnes CA, Franzini-Armstrong C, Gyorke S, Periasamy M. A mutation in calsequestrin, CASQ2D307H, impairs Sarcoplasmic Reticulum Ca^{2+} handling and causes complex ventricular arrhythmias in mice. *Cardiovasc Res*. 2007; 75:69–78. [PubMed: 17449018]
94. Razeghi P, Young ME, Alcorn JL, Moravec CS, Frazier OH, Taegtmeier H. Metabolic gene expression in fetal and failing human heart. *Circulation*. 2001; 104:2923–2931. [PubMed: 11739307]
95. Xu XQ, Soo SY, Sun W, Zweigerdt R. Global expression profile of highly enriched cardiomyocytes derived from human embryonic stem cells. *Stem Cells*. 2009; 27:2163–2174. [PubMed: 19658189]
96. Harris DE, Work SS, Wright RK, Alpert NR, Warshaw DM. Smooth, cardiac and skeletal muscle myosin force and motion generation assessed by cross-bridge mechanical interactions in vitro. *J Muscle Res Cell Motil*. 1994; 15:11–19. [PubMed: 8182105]
97. Holubarsch C, Goulette RP, Litten RZ, Martin BJ, Mulieri LA, Alpert NR. The economy of isometric force development, myosin isoenzyme pattern and myofibrillar ATPase activity in normal and hypothyroid rat myocardium. *Circ Res*. 1985; 56:78–86. [PubMed: 3155672]

98. Sugiura S, Kobayakawa N, Fujita H, Yamashita H, Momomura S, Chaen S, Omata M, Sugi H. Comparison of unitary displacements and forces between 2 cardiac myosin isoforms by the optical trap technique: molecular basis for cardiac adaptation. *Circ Res.* 1998; 82:1029–1034. [PubMed: 9622155]
99. Nakao K, Minobe W, Roden R, Bristow MR, Leinwand LA. Myosin heavy chain gene expression in human heart failure. *J Clin Invest.* 1997; 100:2362–2370. [PubMed: 9410916]
100. Reiser PJ, Portman MA, Ning XH, Schomisch Moravec C. Human cardiac myosin heavy chain isoforms in fetal and failing adult atria and ventricles. *Am J Physiol Heart Circ Physiol.* 2001; 280:H1814–20. [PubMed: 11247796]
101. Sasse S, Brand NJ, Kyprianou P, Dhoot GK, Wade R, Arai M, Periasamy M, Yacoub MH, Barton PJ. Troponin I gene expression during human cardiac development and in end-stage heart failure. *Circ Res.* 1993; 72:932–938. [PubMed: 8477526]
102. Yu ZY, Tan JC, McMahon AC, Iismaa SE, Xiao XH, Kesteven SH, Reichelt ME, Mohl MC, Smith NJ, Fatkin D, Allen D, Head SI, Graham RM, Feneley MP. RhoA/ROCK signaling and pleiotropic α 1A-adrenergic receptor regulation of cardiac contractility. *PLoS One.* 2014; 9:e99024. [PubMed: 24919197]
103. Chuva de Sousa Lopes SM, Hassink RJ, Feijen A, van Rooijen MA, Doevendans PA, Tertoolen L, Brutel de la Riviere A, Mummery CL. Patterning the heart, a template for human cardiomyocyte development. *Dev Dyn.* 2006; 235:1994–2002. [PubMed: 16649168]
104. Schwartz K, Boheler KR, de la Bastie D, Lompre AM, Mercadier JJ. Switches in cardiac muscle gene expression as a result of pressure and volume overload. *Am J Physiol.* 1992; 262:R364–9. [PubMed: 1532697]
105. Rubattu S, Volpe M. The atrial natriuretic peptide: a changing view. *J Hypertens.* 2001; 19:1923–1931. [PubMed: 11677356]
106. Abell TJ, Richards AM, Ikram H, Espiner EA, Yandle T. Atrial natriuretic factor inhibits proliferation of vascular smooth muscle cells stimulated by platelet-derived growth factor. *Biochem Biophys Res Commun.* 1989; 160:1392–1396. [PubMed: 2525038]
107. Horio T, Nishikimi T, Yoshihara F, Matsuo H, Takishita S, Kangawa K. Inhibitory regulation of hypertrophy by endogenous atrial natriuretic peptide in cultured cardiac myocytes. *Hypertension.* 2000; 35:19–24. [PubMed: 10642269]
108. Cao L, Gardner DG. Natriuretic peptides inhibit DNA synthesis in cardiac fibroblasts. *Hypertension.* 1995; 25:227–234. [PubMed: 7843772]
109. Johnson DD, Tetzke TA, Cheung CY. Gene expression of atrial natriuretic factor in ovine fetal heart during development. *J Soc Gynecol Investig.* 1994; 1:14–18.
110. Takahashi T, Allen PD, Izumo S. Expression of A-, B-, and C-type natriuretic peptide genes in failing and developing human ventricles. Correlation with expression of the Ca^{2+} -ATPase gene. *Circ Res.* 1992; 71:9–17. [PubMed: 1535030]
111. Gardner DG, Hedges BK, Wu J, LaPointe MC, Deschepper CF. Expression of the atrial natriuretic peptide gene in human fetal heart. *J Clin Endocrinol Metab.* 1989; 69:729–737. [PubMed: 2528567]
112. Ruskoaho H. Atrial natriuretic peptide: synthesis, release, and metabolism. *Pharmacol Rev.* 1992; 44:479–602. [PubMed: 1480667]
113. Wei YF, Rodi CP, Day ML, Wiegand RC, Needleman LD, Cole BR, Needleman P. Developmental changes in the rat atriopeptin hormonal system. *J Clin Invest.* 1987; 79:1325–1329. [PubMed: 2952670]
114. Scott JN, Jennes L. Distribution of atrial natriuretic factor in fetal rat atria and ventricles. *Cell Tissue Res.* 1987; 248:479–481. [PubMed: 2953427]
115. Hersey RM, Nazir MA, Whitney KD, Klein RM, Sale RD, Hinton DA, Weisz J, Gattone VH 2nd. Atrial natriuretic peptide in heart and specific binding in organs from fetal and newborn rats. *Cell Biochem Funct.* 1989; 7:35–41. [PubMed: 2546691]
116. Iemitsu M, Miyauchi T, Maeda S, Sakai S, Kobayashi T, Fujii N, Miyazaki H, Matsuda M, Yamaguchi I. Physiological and pathological cardiac hypertrophy induce different molecular phenotypes in the rat. *Am J Physiol Regul Integr Comp Physiol.* 2001; 281:R2029–36. [PubMed: 11705790]

117. Yang Z, Yamazaki M, Shen QW, Swartz DR. Differences between cardiac and skeletal troponin interaction with the thin filament probed by troponin exchange in skeletal myofibrils. *Biophys J*. 2009; 97:183–194. [PubMed: 19580756]
118. Thompson BR, Houang EM, Sham YY, Metzger JM. Molecular determinants of cardiac myocyte performance as conferred by isoform-specific TnI residues. *Biophys J*. 2014; 106:2105–2114. [PubMed: 24853739]
119. Mahmoud AI, Kocabas F, Muralidhar SA, Kimura W, Koura AS, Thet S, Porrello ER, Sadek HA. Meis1 regulates postnatal cardiomyocyte cell cycle arrest. *Nature*. 2013; 497:249–253. [PubMed: 23594737]
120. Porrello ER, Mahmoud AI, Simpson E, Hill JA, Richardson JA, Olson EN, Sadek HA. Transient regenerative potential of the neonatal mouse heart. *Science*. 2011; 331:1078–1080. [PubMed: 21350179]
121. Porrello ER, Mahmoud AI, Simpson E, Johnson BA, Grinsfelder D, Canseco D, Mammen PP, Rothmel BA, Olson EN, Sadek HA. Regulation of neonatal and adult mammalian heart regeneration by the miR-15 family. *Proc Natl Acad Sci U S A*. 2013; 110:187–192. [PubMed: 23248315]
122. Xin M, Kim Y, Sutherland LB, Murakami M, Qi X, McAnally J, Porrello ER, Mahmoud AI, Tan W, Shelton JM, Richardson JA, Sadek HA, Bassel-Duby R, Olson EN. Hippo pathway effector Yap promotes cardiac regeneration. *Proc Natl Acad Sci U S A*. 2013; 110:13839–13844. [PubMed: 23918388]
123. Flashman E, Redwood C, Moolman-Smook J, Watkins H. Cardiac myosin binding protein C: its role in physiology and disease. *Circ Res*. 2004; 94:1279–1289. [PubMed: 15166115]
124. Furst DO, Osborn M, Nave R, Weber K. The organization of titin filaments in the half-sarcomere revealed by monoclonal antibodies in immunoelectron microscopy: a map of ten nonrepetitive epitopes starting at the Z line extends close to the M line. *J Cell Biol*. 1988; 106:1563–1572. [PubMed: 2453516]
125. Helmes M, Trombitas K, Centner T, Kellermayer M, Labeit S, Linke WA, Granzier H. Mechanically driven contour-length adjustment in rat cardiac titin's unique N2B sequence: titin is an adjustable spring. *Circ Res*. 1999; 84:1339–1352. [PubMed: 10364572]
126. Trombitas K, Jin JP, Granzier H. The mechanically active domain of titin in cardiac muscle. *Circ Res*. 1995; 77:856–861. [PubMed: 7554133]
127. Trombitas K, Freiburg A, Centner T, Labeit S, Granzier H. Molecular dissection of N2B cardiac titin's extensibility. *Biophys J*. 1999; 77:3189–3196. [PubMed: 10585940]
128. Linke WA, Rudy DE, Centner T, Gautel M, Witt C, Labeit S, Gregorio CC. I-band titin in cardiac muscle is a three-element molecular spring and is critical for maintaining thin filament structure. *J Cell Biol*. 1999; 146:631–644. [PubMed: 10444071]
129. Cazorla O, Freiburg A, Helmes M, Centner T, McNabb M, Wu Y, Trombitas K, Labeit S, Granzier H. Differential expression of cardiac titin isoforms and modulation of cellular stiffness. *Circ Res*. 2000; 86:59–67. [PubMed: 10625306]
130. Greaser ML, Krzesinski PR, Warren CM, Kirkpatrick B, Campbell KS, Moss RL. Developmental changes in rat cardiac titin/connectin: transitions in normal animals and in mutants with a delayed pattern of isoform transition. *J Muscle Res Cell Motil*. 2005; 26:325–332. [PubMed: 16491431]
131. Lahmers S, Wu Y, Call DR, Labeit S, Granzier H. Developmental control of titin isoform expression and passive stiffness in fetal and neonatal myocardium. *Circ Res*. 2004; 94:505–513. [PubMed: 14707027]
132. Opitz CA, Leake MC, Makarenko I, Benes V, Linke WA. Developmentally regulated switching of titin size alters myofibrillar stiffness in the perinatal heart. *Circ Res*. 2004; 94:967–975. [PubMed: 14988228]
133. Bang ML, Centner T, Fornoff F, Geach AJ, Gotthardt M, McNabb M, Witt CC, Labeit D, Gregorio CC, Granzier H, Labeit S. The complete gene sequence of titin, expression of an unusual approximately 700-kDa titin isoform, and its interaction with obscurin identify a novel Z-line to I-band linking system. *Circ Res*. 2001; 89:1065–1072. [PubMed: 11717165]
134. LeWinter MM, Granzier H. Cardiac titin: a multifunctional giant. *Circulation*. 2010; 121:2137–2145. [PubMed: 20479164]

135. Trombitas K, Redkar A, Centner T, Wu Y, Labeit S, Granzier H. Extensibility of isoforms of cardiac titin: variation in contour length of molecular subsegments provides a basis for cellular passive stiffness diversity. *Biophys J*. 2000; 79:3226–3234. [PubMed: 11106626]
136. Fabiato A. Calcium-induced release of calcium from the cardiac sarcoplasmic reticulum. *Am J Physiol*. 1983; 245:C1–14. [PubMed: 6346892]
137. Carafoli E, Santella L, Branca D, Brini M. Generation, control, and processing of cellular calcium signals. *Crit Rev Biochem Mol Biol*. 2001; 36:107–260. [PubMed: 11370791]
138. Farah CS, Reinach FC. The troponin complex and regulation of muscle contraction. *FASEB J*. 1995; 9:755–767. [PubMed: 7601340]
139. Bassani JW, Bassani RA, Bers DM. Relaxation in rabbit and rat cardiac cells: species-dependent differences in cellular mechanisms. *J Physiol*. 1994; 476:279–293. [PubMed: 8046643]
140. Kim C, Majdi M, Xia P, Wei KA, Talantova M, Spiering S, Nelson B, Mercola M, Chen HS. Non-cardiomyocytes influence the electrophysiological maturation of human embryonic stem cell-derived cardiomyocytes during differentiation. *Stem Cells Dev*. 2010; 19:783–795. [PubMed: 20001453]
141. Bers DM. Cardiac excitation-contraction coupling. *Nature*. 2002; 415:198–205. [PubMed: 11805843]
142. Dolnikov K, Shilkrut M, Zeevi-Levin N, Danon A, Gerecht-Nir S, Itskovitz-Eldor J, Binah O. Functional properties of human embryonic stem cell-derived cardiomyocytes. *Ann N Y Acad Sci*. 2005; 1047:66–75. [PubMed: 16093485]
143. Dolnikov K, Shilkrut M, Zeevi-Levin N, Gerecht-Nir S, Amit M, Danon A, Itskovitz-Eldor J, Binah O. Functional properties of human embryonic stem cell-derived cardiomyocytes: intracellular Ca²⁺ handling and the role of sarcoplasmic reticulum in the contraction. *Stem Cells*. 2006; 24:236–245. [PubMed: 16322641]
144. Itzhaki I, Rapoport S, Huber I, Mizrahi I, Zwi-Dantsis L, Arbel G, Schiller J, Gepstein L. Calcium handling in human induced pluripotent stem cell derived cardiomyocytes. *PLoS One*. 2011; 6:e18037. [PubMed: 21483779]
145. Lee YK, Ng KM, Lai WH, Chan YC, Lau YM, Lian Q, Tse HF, Siu CW. Calcium homeostasis in human induced pluripotent stem cell-derived cardiomyocytes. *Stem Cell Rev*. 2011; 7:976–986. [PubMed: 21614516]
146. Liu J, Lieu DK, Siu CW, Fu JD, Tse HF, Li RA. Facilitated maturation of Ca²⁺ handling properties of human embryonic stem cell-derived cardiomyocytes by calsequestrin expression. *Am J Physiol Cell Physiol*. 2009; 297:C152–9. [PubMed: 19357236]
147. Nakanishi T, Seguchi M, Takao A. Development of the myocardial contractile system. *Experientia*. 1988; 44:936–944. [PubMed: 3058500]
148. Pegg W, Michalak M. Differentiation of sarcoplasmic reticulum during cardiac myogenesis. *Am J Physiol*. 1987; 252:H22–31. [PubMed: 3028162]
149. Kang J, Chen XL, Ji J, Lei Q, Rampe D. Ca(2)(+) channel activators reveal differential L-type Ca(2)(+) channel pharmacology between native and stem cell-derived cardiomyocytes. *J Pharmacol Exp Ther*. 2012; 341:510–517. [PubMed: 22353878]
150. Binah O, Dolnikov K, Sadan O, Shilkrut M, Zeevi-Levin N, Amit M, Danon A, Itskovitz-Eldor J. Functional and developmental properties of human embryonic stem cells-derived cardiomyocytes. *J Electrocardiol*. 2007; 40:S192–6. [PubMed: 17993321]
151. Fu JD, Li J, Tweedie D, Yu HM, Chen L, Wang R, Riordon DR, Brugh SA, Wang SQ, Boheler KR, Yang HT. Crucial role of the sarcoplasmic reticulum in the developmental regulation of Ca²⁺ transients and contraction in cardiomyocytes derived from embryonic stem cells. *FASEB J*. 2006; 20:181–183. [PubMed: 16249315]
152. Xi J, Khalil M, Shishechian N, Hannes T, Pfannkuche K, Liang H, Fatima A, Hausteim M, Suhr F, Bloch W, Reppel M, Saric T, Wernig M, Janisch R, Brockmeier K, Hescheler J, Pillekamp F. Comparison of contractile behavior of native murine ventricular tissue and cardiomyocytes derived from embryonic or induced pluripotent stem cells. *FASEB J*. 2010; 24:2739–2751. [PubMed: 20371616]
153. Doss MX, Di Diego JM, Goodrow RJ, Wu Y, Cordeiro JM, Nesterenko VV, Barajas-Martinez H, Hu D, Urrutia J, Desai M, Treat JA, Sachinidis A, Antzelevitch C. Maximum diastolic potential

of human induced pluripotent stem cell-derived cardiomyocytes depends critically on I(Kr). *PLoS One*. 2012; 7:e40288. [PubMed: 22815737]

154. Koncz I, Szel T, Bitay M, Cerbai E, Jaeger K, Fulop F, Jost N, Virag L, Orvos P, Talosi L, Kristof A, Bacsko I, Papp JG, Varro A. Electrophysiological effects of ivabradine in dog and human cardiac preparations: potential antiarrhythmic actions. *Eur J Pharmacol*. 2011; 668:419–426. [PubMed: 21821019]
155. Drouin E, Charpentier F, Gauthier C, Laurent K, Le Marec H. Electrophysiologic characteristics of cells spanning the left ventricular wall of human heart: evidence for presence of M cells. *J Am Coll Cardiol*. 1995; 26:185–192. [PubMed: 7797750]
156. Gennser G, Nilsson E. Excitation and impulse conduction in the human fetal heart. *Acta Physiol Scand*. 1970; 79:305–320. [PubMed: 5450407]
157. Thompson SA, Burrige PW, Lipke EA, Shablott M, Zambidis ET, Tung L. Engraftment of human embryonic stem cell derived cardiomyocytes improves conduction in an arrhythmogenic in vitro model. *J Mol Cell Cardiol*. 2012; 53:15–23. [PubMed: 22713758]
158. Sheng X, Reppel M, Nguemo F, Mohammad FI, Kuzmenkin A, Hescheler J, Pfannkuche K. Human pluripotent stem cell-derived cardiomyocytes: response to TTX and lidocaine reveals strong cell to cell variability. *PLoS One*. 2012; 7:e45963. [PubMed: 23029342]
159. Zhu WZ, Xie Y, Moyes KW, Gold JD, Askari B, Laflamme MA. Neuregulin/ErbB signaling regulates cardiac subtype specification in differentiating human embryonic stem cells. *Circ Res*. 2010; 107:776–786. [PubMed: 20671236]
160. Polak S, Fijorek K. Inter-individual variability in the pre-clinical drug cardiotoxic safety assessment--analysis of the age-cardiomyocytes electric capacitance dependence. *J Cardiovasc Transl Res*. 2012; 5:321–332. [PubMed: 22411323]
161. Liao B, Zhang D, Bursac N. Functional cardiac tissue engineering. *Regen Med*. 2012; 7:187–206. [PubMed: 22397609]
162. Weiss JN, Nivala M, Garfinkel A, Qu Z. Alternans and arrhythmias: from cell to heart. *Circ Res*. 2011; 108:98–112. [PubMed: 21212392]
163. Smith JM, Clancy EA, Valeri CR, Ruskin JN, Cohen RJ. Electrical alternans and cardiac electrical instability. *Circulation*. 1988; 77:110–121. [PubMed: 3335062]
164. Seed WA, Noble MI, Oldershaw P, Wanless RB, Drake-Holland AJ, Redwood D, Pugh S, Mills C. Relation of human cardiac action potential duration to the interval between beats: implications for the validity of rate corrected QT interval (QTc). *Br Heart J*. 1987; 57:32–37. [PubMed: 3801256]
165. Rosenbaum DS, Jackson LE, Smith JM, Garan H, Ruskin JN, Cohen RJ. Electrical alternans and vulnerability to ventricular arrhythmias. *N Engl J Med*. 1994; 330:235–241. [PubMed: 8272084]
166. Nolasco JB, Dahlen RW. A graphic method for the study of alternation in cardiac action potentials. *J Appl Physiol*. 1968; 25:191–196. [PubMed: 5666097]
167. van Leeuwen P, Schiermeier S, Lange S, Klein A, Geue D, Hatzmann W, Gronemeyer DH. Gender-related changes in magnetocardiographically determined fetal cardiac time intervals in intrauterine growth retardation. *Pediatr Res*. 2006; 59:820–824. [PubMed: 16641208]
168. Hayashi H, Shiferaw Y, Sato D, Nihei M, Lin SF, Chen PS, Garfinkel A, Weiss JN, Qu Z. Dynamic origin of spatially discordant alternans in cardiac tissue. *Biophys J*. 2007; 92:448–460. [PubMed: 17071663]
169. Bers DM, Berlin JR. Kinetics of [Ca]_i decline in cardiac myocytes depend on peak [Ca]_i. *Am J Physiol*. 1995; 268:C271–7. [PubMed: 7840156]
170. Clark RB, Bouchard RA, Giles WR. Action potential duration modulates calcium influx, Na(+)-Ca²⁺ exchange, and intracellular calcium release in rat ventricular myocytes. *Ann N Y Acad Sci*. 1996; 779:417–429. [PubMed: 8659858]
171. Cooper IC, Fry CH. Mechanical restitution in isolated mammalian myocardium: species differences and underlying mechanisms. *J Mol Cell Cardiol*. 1990; 22:439–452. [PubMed: 2388277]
172. Cao F, Wagner RA, Wilson KD, Xie X, Fu JD, Drukker M, Lee A, Li RA, Gambhir SS, Weissman IL, Robbins RC, Wu JC. Transcriptional and functional profiling of human embryonic stem cell-derived cardiomyocytes. *PLoS One*. 2008; 3:e3474. [PubMed: 18941512]

173. Jackman CP, Shadrin IY, Carlson AL, Bursac N. Human Cardiac Tissue Engineering: From Pluripotent Stem Cells to Heart Repair. *Curr Opin Chem Eng.* 2015; 7:57–64. [PubMed: 25599018]
174. Jacobson SL, Piper HM. Cell cultures of adult cardiomyocytes as models of the myocardium. *J Mol Cell Cardiol.* 1986; 18:661–678. [PubMed: 3528506]
175. Brito-Martins M, Harding SE, Ali NN. Beta(1)- and Beta(2)-Adrenoceptor Responses in Cardiomyocytes Derived from Human Embryonic Stem Cells: Comparison with Failing and Non-Failing Adult Human Heart. *Br J Pharmacol.* 2008; 153:751–759. [PubMed: 18193079]
176. Zhang J, Wilson GF, Soerens AG, Koonce CH, Yu J, Palecek SP, Thomson JA, Kamp TJ. Functional cardiomyocytes derived from human induced pluripotent stem cells. *Circ Res.* 2009; 104:e30–41. [PubMed: 19213953]
177. Xu RH, Chen X, Li DS, Li R, Addicks GC, Glennon C, Zwaka TP, Thomson JA. BMP4 initiates human embryonic stem cell differentiation to trophoblast. *Nat Biotechnol.* 2002; 20:1261–1264. [PubMed: 12426580]
178. Reppel M, Boettinger C, Hescheler J. Beta-adrenergic and muscarinic modulation of human embryonic stem cell-derived cardiomyocytes. *Cell Physiol Biochem.* 2004; 14:187–196. [PubMed: 15319521]
179. Caspi O, Lesman A, Basevitch Y, Gepstein A, Arbel G, Habib IH, Gepstein L, Levenberg S. Tissue engineering of vascularized cardiac muscle from human embryonic stem cells. *Circ Res.* 2007; 100:263–272. [PubMed: 17218605]
180. Jongsma HJ, Wilders R. Gap junctions in cardiovascular disease. *Circ Res.* 2000; 86:1193–1197. [PubMed: 10864907]
181. Friedman WF. The intrinsic physiologic properties of the developing heart. *Prog Cardiovasc Dis.* 1972; 15:87–111. [PubMed: 4402451]
182. Nassar R, Reedy MC, Anderson PA. Developmental changes in the ultrastructure and sarcomere shortening of the isolated rabbit ventricular myocyte. *Circ Res.* 1987; 61:465–483. [PubMed: 2441892]
183. van der Velden J, Klein LJ, van der Bijl M, Huybregts MA, Stooker W, Witkop J, Eijssman L, Visser CA, Visser FC, Stienen GJ. Force production in mechanically isolated cardiac myocytes from human ventricular muscle tissue. *Cardiovasc Res.* 1998; 38:414–423. [PubMed: 9709402]
184. Wiegerinck RF, Cojoc A, Zeidenweber CM, Ding G, Shen M, Joyner RW, Fernandez JD, Kanter KR, Kirshbom PM, Kogon BE, Wagner MB. Force frequency relationship of the human ventricle increases during early postnatal development. *Pediatr Res.* 2009; 65:414–419. [PubMed: 19127223]
185. Hazeltine LB, Simmons CS, Salick MR, Lian X, Badur MG, Han W, Delgado SM, Wakatsuki T, Crone WC, Pruitt BL, Palecek SP. Effects of substrate mechanics on contractility of cardiomyocytes generated from human pluripotent stem cells. *Int J Cell Biol.* 2012; 2012:508294. [PubMed: 22649451]
186. Kita-Matsuo H, Barcova M, Prigozhina N, Salomonis N, Wei K, Jacot JG, Nelson B, Spiering S, Haverslag R, Kim C, Talantova M, Bajpai R, Calzolari D, Terskikh A, McCulloch AD, Price JH, Conklin BR, Chen HS, Mercola M. Lentiviral vectors and protocols for creation of stable hESC lines for fluorescent tracking and drug resistance selection of cardiomyocytes. *PLoS One.* 2009; 4:e5046. [PubMed: 19352491]
187. Lee EJ, Peng J, Radke M, Gotthardt M, Granzier HL. Calcium sensitivity and the Frank-Starling mechanism of the heart are increased in titin N2B region-deficient mice. *J Mol Cell Cardiol.* 2010; 49:449–458. [PubMed: 20507834]
188. Ursem NT, Struijk PC, Hop WC, Clark EB, Keller BB, Wladimiroff JW. Heart rate and flow velocity variability as determined from umbilical Doppler velocimetry at 10–20 weeks of gestation. *Clin Sci (Lond).* 1998; 95:539–545. [PubMed: 9791039]
189. Germanguz I, Sedan O, Zeevi-Levin N, Shtrichman R, Barak E, Ziskind A, Eliyahu S, Meiry G, Amit M, Itskovitz-Eldor J, Binah O. Molecular characterization and functional properties of cardiomyocytes derived from human inducible pluripotent stem cells. *J Cell Mol Med.* 2011; 15:38–51. [PubMed: 20041972]

190. Xu C, Police S, Rao N, Carpenter MK. Characterization and enrichment of cardiomyocytes derived from human embryonic stem cells. *Circ Res.* 2002; 91:501–508. [PubMed: 12242268]
191. Cain BS, Meldrum DR, Meng X, Shames BD, Banerjee A, Harken AH. Calcium preconditioning in human myocardium. *Ann Thorac Surg.* 1998; 65:1065–1070. [PubMed: 9564929]
192. Streckfuss-Bomeke K, Wolf F, Azizian A, Stauske M, Tiburcy M, Wagner S, Hubscher D, Dressel R, Chen S, Jende J, Wulf G, Lorenz V, Schon MP, Maier LS, Zimmermann WH, Hasenfuss G, Guan K. Comparative study of human-induced pluripotent stem cells derived from bone marrow cells, hair keratinocytes, and skin fibroblasts. *Eur Heart J.* 2013; 34:2618–2629. [PubMed: 22798560]
193. Turnbull IC, Karakikes I, Serrao GW, Backeris P, Lee JJ, Xie C, Senyei G, Gordon RE, Li RA, Akar FG, Hajjar RJ, Hulot JS, Costa KD. Advancing functional engineered cardiac tissues toward a preclinical model of human myocardium. *FASEB J.* 2014; 28:644–654. [PubMed: 24174427]
194. Kranias EG, Solaro RJ. Phosphorylation of troponin I and phospholamban during catecholamine stimulation of rabbit heart. *Nature.* 1982; 298:182–184. [PubMed: 6211626]
195. Brandenburger M, Wenzel J, Bogdan R, Richardt D, Nguemo F, Reppel M, Hescheler J, Terlau H, Dendorfer A. Organotypic slice culture from human adult ventricular myocardium. *Cardiovasc Res.* 2012; 93:50–59. [PubMed: 21972180]
196. Ramirez-Correa GA, Murphy AM. Is phospholamban or troponin I the “prima donna” in beta-adrenergic induced lusitropy? *Circ Res.* 2007; 101:326–327. [PubMed: 17702976]
197. MacLennan DH, Asahi M, Tupling AR. The regulation of SERCA-type pumps by phospholamban and sarcolipin. *Ann N Y Acad Sci.* 2003; 986:472–480. [PubMed: 12763867]
198. Eschenhagen T, Eder A, Vollert I, Hansen A. Physiological aspects of cardiac tissue engineering. *Am J Physiol Heart Circ Physiol.* 2012; 303:H133–43. [PubMed: 22582087]
199. Flesch M, Schwinger RH, Schiffer F, Frank K, Sudkamp M, Kuhn-Regnier F, Arnold G, Bohm M. Evidence for functional relevance of an enhanced expression of the Na(+)-Ca2+ exchanger in failing human myocardium. *Circulation.* 1996; 94:992–1002. [PubMed: 8790037]
200. Holubarsch C, Schneider R, Pieske B, Ruf T, Hasenfuss G, Fraedrich G, Posival H, Just H. Positive and negative inotropic effects of DL-sotalol and D-sotalol in failing and nonfailing human myocardium under physiological experimental conditions. *Circulation.* 1995; 92:2904–2910. [PubMed: 7586258]
201. Bohm M, Morano I, Pieske B, Ruegg JC, Wankel M, Zimmermann R, Erdmann E. Contribution of cAMP-phosphodiesterase inhibition and sensitization of the contractile proteins for calcium to the inotropic effect of pimobendan in the failing human myocardium. *Circ Res.* 1991; 68:689–701. [PubMed: 1660359]
202. White M, Roden R, Minobe W, Khan MF, Larrabee P, Wollmering M, Port JD, Anderson F, Campbell D, Feldman AM. Age-related changes in beta-adrenergic neuroeffector systems in the human heart. *Circulation.* 1994; 90:1225–1238. [PubMed: 8087932]
203. Biegon RL, Pappano AJ. Dual mechanism for inhibition of calcium-dependent action potentials by acetylcholine in avian ventricular muscle. Relationship to cyclic AMP. *Circ Res.* 1980; 46:353–362. [PubMed: 6244118]
204. Pappano AJ, Hartigan PM, Coutu MD. Acetylcholine inhibits positive inotropic effect of cholera toxin in ventricular muscle. *Am J Physiol.* 1982; 243:H434–41. [PubMed: 6287867]
205. Von Scheidt W, Bohm M, Stablein A, Autenrieth G, Erdmann E. Antiadrenergic effect of M-cholinoceptor stimulation on human ventricular contractility in vivo. *Am J Physiol.* 1992; 263:H1927–31. [PubMed: 1336317]
206. Koglin J, Bohm M, von Scheidt W, Stablein A, Erdmann E. Antiadrenergic effect of carbachol but not of adenosine on contractility in the intact human ventricle in vivo. *J Am Coll Cardiol.* 1994; 23:678–683. [PubMed: 8113552]
207. Zhang S, Zhou Z, Gong Q, Makielski JC, January CT. Mechanism of block and identification of the verapamil binding domain to HERG potassium channels. *Circ Res.* 1999; 84:989–998. [PubMed: 10325236]

208. Harris K, Aylott M, Cui Y, Louttit JB, McMahon NC, Sridhar A. Comparison of electrophysiological data from human-induced pluripotent stem cell-derived cardiomyocytes to functional preclinical safety assays. *Toxicol Sci.* 2013; 134:412–426. [PubMed: 23690542]
209. Freeze BS, McNulty MM, Hanck DA. State-dependent verapamil block of the cloned human Ca(v)3.1 T-type Ca(2+) channel. *Mol Pharmacol.* 2006; 70:718–726. [PubMed: 16699084]
210. Kuryshv YA, Brown AM, Duzic E, Kirsch GE. Evaluating state dependence and subtype selectivity of calcium channel modulators in automated electrophysiology assays. *Assay Drug Dev Technol.* 2014; 12:110–119. [PubMed: 24579774]
211. Yamazaki K, Hihara T, Kato H, Fukushima T, Fukushima K, Taniguchi T, Yoshinaga T, Miyamoto N, Ito M, Sawada K. Beat-to-Beat Variability in Field Potential Duration in Human Embryonic Stem Cell-Derived Cardiomyocyte Clusters for Assessment of Arrhythmogenic Risk, and a Case Study of Its Application. *Pharmacology and Pharmacy.* 2014; 5:117–128.
212. Sarsero D, Fujiwara T, Molenaar P, Angus JA. Human vascular to cardiac tissue selectivity of L- and T-type calcium channel antagonists. *Br J Pharmacol.* 1998; 125:109–119. [PubMed: 9776350]
213. Guo L, Abrams RM, Babiarz JE, Cohen JD, Kameoka S, Sanders MJ, Chiao E, Kolaja KL. Estimating the risk of drug-induced proarrhythmia using human induced pluripotent stem cell-derived cardiomyocytes. *Toxicol Sci.* 2011; 123:281–289. [PubMed: 21693436]
214. Gibson JK, Yue Y, Bronson J, Palmer C, Numann R. Human stem cell-derived cardiomyocytes detect drug-mediated changes in action potentials and ion currents. *J Pharmacol Toxicol Methods.* 2014; 70:255–267. [PubMed: 25219538]
215. Splawski I, Timothy KW, Sharpe LM, Decher N, Kumar P, Bloise R, Napolitano C, Schwartz PJ, Joseph RM, Condouris K, Tager-Flusberg H, Priori SG, Sanguinetti MC, Keating MT. Ca(V)1.2 calcium channel dysfunction causes a multisystem disorder including arrhythmia and autism. *Cell.* 2004; 119:19–31. [PubMed: 15454078]
216. Liu J, Fu JD, Siu CW, Li RA. Functional sarcoplasmic reticulum for calcium handling of human embryonic stem cell-derived cardiomyocytes: insights for driven maturation. *Stem Cells.* 2007; 25:3038–3044. [PubMed: 17872499]
217. Poindexter BJ, Smith JR, Buja LM, Bick RJ. Calcium signaling mechanisms in dedifferentiated cardiac myocytes: comparison with neonatal and adult cardiomyocytes. *Cell Calcium.* 2001; 30:373–382. [PubMed: 11728132]
218. Zhu WZ, Santana LF, Laflamme MA. Local control of excitation-contraction coupling in human embryonic stem cell-derived cardiomyocytes. *PLoS One.* 2009; 4:e5407. [PubMed: 19404384]
219. Fill M, Copello JA. Ryanodine receptor calcium release channels. *Physiol Rev.* 2002; 82:893–922. [PubMed: 12270947]
220. Roy M, Dumaine R, Brown AM. HERG, a primary human ventricular target of the non-sedating antihistamine terfenadine. *Circulation.* 1996; 94:817–823. [PubMed: 8772706]
221. Guo L, Qian JY, Abrams R, Tang HM, Weiser T, Sanders MJ, Kolaja KL. The electrophysiological effects of cardiac glycosides in human iPSC-derived cardiomyocytes and in guinea pig isolated hearts. *Cell Physiol Biochem.* 2011; 27:453–462. [PubMed: 21691062]
222. Ming Z, Nordin C. Terfenadine blocks time-dependent Ca²⁺, Na⁺, and K⁺ channels in guinea pig ventricular myocytes. *J Cardiovasc Pharmacol.* 1995; 26:761–769. [PubMed: 8637191]
223. Lu HR, Hermans AN, Gallacher DJ. Does terfenadine-induced ventricular tachycardia/fibrillation directly relate to its QT prolongation and Torsades de Pointes? *Br J Pharmacol.* 2012; 166:1490–1502. [PubMed: 22300168]
224. Hove-Madsen L, Llach A, Molina CE, Prat-Vidal C, Farre J, Roura S, Cinca J. The proarrhythmic antihistaminic drug terfenadine increases spontaneous calcium release in human atrial myocytes. *Eur J Pharmacol.* 2006; 553:215–221. [PubMed: 17078945]
225. Lacerda AE, Kramer J, Shen KZ, Thomas D, Brown AM. Comparison of block among cloned cardiac potassium channels by non-antiarrhythmic drugs. *European Heart Journal.* 2001; 3:K23–K30.
226. Baruscotti M, Bucchi A, Difrancesco D. Physiology and pharmacology of the cardiac pacemaker (“funny”) current. *Pharmacol Ther.* 2005; 107:59–79. [PubMed: 15963351]

227. Ju YK, Gage PW, Saint DA. Tetrodotoxin-sensitive inactivation-resistant sodium channels in pacemaker cells influence heart rate. *Pflugers Arch*. 1996; 431:868–875. [PubMed: 8927503]
228. Catterall WA, Goldin AL, Waxman SG. International Union of Pharmacology. XLVII. Nomenclature and structure-function relationships of voltage-gated sodium channels. *Pharmacol Rev*. 2005; 57:397–409. [PubMed: 16382098]
229. Goldin AL. Resurgence of sodium channel research. *Annu Rev Physiol*. 2001; 63:871–894. [PubMed: 11181979]
230. Barandi L, Virag L, Jost N, Horvath Z, Koncz I, Papp R, Harmati G, Horvath B, Szentandrassy N, Banyasz T, Magyar J, Zaza A, Varro A, Nanasi PP. Reverse rate-dependent changes are determined by baseline action potential duration in mammalian and human ventricular preparations. *Basic Res Cardiol*. 2010; 105:315–323. [PubMed: 20127488]
231. Bennett PB, Valenzuela C, Chen LQ, Kallen RG. On the molecular nature of the lidocaine receptor of cardiac Na⁺ channels. Modification of block by alterations in the alpha-subunit III–IV interdomain. *Circ Res*. 1995; 77:584–592. [PubMed: 7641328]
232. Kane KA. Comparative electrophysiological effects of Org 6001, a new orally active antidysrhythmic agent, and lignocaine on human ventricular muscle. *Br J Pharmacol*. 1980; 68:25–31. [PubMed: 7357199]
233. Mehta A, Chung Y, Sequiera GL, Wong P, Liew R, Shim W. Pharmacoelectrophysiology of viral-free induced pluripotent stem cell-derived human cardiomyocytes. *Toxicol Sci*. 2013; 131:458–469. [PubMed: 23091167]
234. Thavandiran N, Dubois N, Mikryukov A, Masse S, Beca B, Simmons CA, Deshpande VS, McGarry JP, Chen CS, Nanthakumar K, Keller GM, Radisic M, Zandstra PW. Design and formulation of functional pluripotent stem cell-derived cardiac microtissues. *Proc Natl Acad Sci U S A*. 2013; 110:E4698–707. [PubMed: 24255110]
235. Jacot JG, McCulloch AD, Omens JH. Substrate stiffness affects the functional maturation of neonatal rat ventricular myocytes. *Biophys J*. 2008; 95:3479–3487. [PubMed: 18586852]
236. Kuo PL, Lee H, Bray MA, Geisse NA, Huang YT, Adams WJ, Sheehy SP, Parker KK. Myocyte shape regulates lateral registry of sarcomeres and contractility. *Am J Pathol*. 2012; 181:2030–2037. [PubMed: 23159216]
237. Xia Y, Buja LM, Scarpulla RC, McMillin JB. Electrical stimulation of neonatal cardiomyocytes results in the sequential activation of nuclear genes governing mitochondrial proliferation and differentiation. *Proc Natl Acad Sci U S A*. 1997; 94:11399–11404. [PubMed: 9326621]
238. Mihic A, Li J, Miyagi Y, Gagliardi M, Li SH, Zu J, Weisel RD, Keller G, Li RK. The effect of cyclic stretch on maturation and 3D tissue formation of human embryonic stem cell-derived cardiomyocytes. *Biomaterials*. 2014; 35:2798–2808. [PubMed: 24424206]
239. Kim C, Wong J, Wen J, Wang S, Wang C, Spiering S, Kan NG, Forcales S, Puri PL, Leone TC, Marine JE, Calkins H, Kelly DP, Judge DP, Chen HS. Studying arrhythmogenic right ventricular dysplasia with patient-specific iPSCs. *Nature*. 2013; 494:105–110. [PubMed: 23354045]
240. Zimmermann WH, Schneiderbanger K, Schubert P, Didie M, Munzel F, Heubach JF, Kostin S, Neuhuber WL, Eschenhagen T. Tissue engineering of a differentiated cardiac muscle construct. *Circ Res*. 2002; 90:223–230. [PubMed: 11834716]
241. Nunes SS, Miklas JW, Liu J, Aschar-Sobbi R, Xiao Y, Zhang B, Jiang J, Masse S, Gagliardi M, Hsieh A, Thavandiran N, Laflamme MA, Nanthakumar K, Gross GJ, Backx PH, Keller G, Radisic M. Biowire: a platform for maturation of human pluripotent stem cell-derived cardiomyocytes. *Nat Methods*. 2013; 10:781–787. [PubMed: 23793239]
242. Hirt MN, Boedinghaus J, Mitchell A, Schaaf S, Bornchen C, Muller C, Schulz H, Hubner N, Stenzig J, Stoehr A, Neuber C, Eder A, Luther PK, Hansen A, Eschenhagen T. Functional improvement and maturation of rat and human engineered heart tissue by chronic electrical stimulation. *J Mol Cell Cardiol*. 2014; 74:151–161. [PubMed: 24852842]
243. Zhang D, Shadrin IY, Lam J, Xian HQ, Snodgrass HR, Bursac N. Tissue-engineered cardiac patch for advanced functional maturation of human ESC-derived cardiomyocytes. *Biomaterials*. 2013; 34:5813–5820. [PubMed: 23642535]

244. Ott HC, Matthiesen TS, Goh SK, Black LD, Kren SM, Netoff TI, Taylor DA. Perfusion-decellularized matrix: using nature's platform to engineer a bioartificial heart. *Nat Med.* 2008; 14:213–221. [PubMed: 18193059]
245. Cambier L, Plate M, Sucov HM, Pashmforoush M. Nkx2–5 regulates cardiac growth through modulation of Wnt signaling by R-spondin3. *Development.* 2014; 141:2959–2971. [PubMed: 25053429]
246. Carlsson L. In vitro and in vivo models for testing arrhythmogenesis in drugs. *J Intern Med.* 2006; 259:70–80. [PubMed: 16336515]
247. Liang H, Matzkies M, Schunkert H, Tang M, Bonnemeier H, Hescheler J, Reppel M. Human and murine embryonic stem cell-derived cardiomyocytes serve together as a valuable model for drug safety screening. *Cell Physiol Biochem.* 2010; 25:459–466. [PubMed: 20332627]
248. Peng S, Lacerda AE, Kirsch GE, Brown AM, Bruening-Wright A. The action potential and comparative pharmacology of stem cell-derived human cardiomyocytes. *J Pharmacol Toxicol Methods.* 2010; 61:277–286. [PubMed: 20153443]
249. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell.* 2007; 131:861–872. [PubMed: 18035408]
250. Carvajal-Vergara X, Sevilla A, D'Souza SL, Ang YS, Schaniel C, Lee DF, Yang L, Kaplan AD, Adler ED, Rozov R, Ge Y, Cohen N, Edelmann LJ, Chang B, Waghay A, Su J, Pardo S, Lichtenbelt KD, Tartaglia M, Gelb BD, Lemischka IR. Patient-specific induced pluripotent stem-cell-derived models of LEOPARD syndrome. *Nature.* 2010; 465:808–812. [PubMed: 20535210]
251. 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. Modelling the long QT syndrome with induced pluripotent stem cells. *Nature.* 2011; 471:225–229. [PubMed: 21240260]
252. Zwi-Dantsis L, Huber I, Habib M, Winterstern A, Gepstein A, Arbel G, Gepstein L. Derivation and cardiomyocyte differentiation of induced pluripotent stem cells from heart failure patients. *Eur Heart J.* 2013; 34:1575–1586. [PubMed: 22621821]
253. Aasen T, Raya A, Barrero MJ, Garreta E, Consiglio A, Gonzalez F, Vassena R, Bilic J, Pekarik V, Tiscornia G, Edel M, Boue S, Izpisua Belmonte JC. Efficient and rapid generation of induced pluripotent stem cells from human keratinocytes. *Nat Biotechnol.* 2008; 26:1276–1284. [PubMed: 18931654]
254. Loh YH, Agarwal S, Park IH, Urbach A, Huo H, Heffner GC, Kim K, Miller JD, Ng K, Daley GQ. Generation of induced pluripotent stem cells from human blood. *Blood.* 2009; 113:5476–5479. [PubMed: 19299331]
255. Sun N, Panetta NJ, Gupta DM, Wilson KD, Lee A, Jia F, Hu S, Cherry AM, Robbins RC, Longaker MT, Wu JC. Feeder-free derivation of induced pluripotent stem cells from adult human adipose stem cells. *Proc Natl Acad Sci U S A.* 2009; 106:15720–15725. [PubMed: 19805220]
256. Tamaoki N, Takahashi K, Tanaka T, Ichisaka T, Aoki H, Takeda-Kawaguchi T, Iida K, Kunisada T, Shibata T, Yamanaka S, Tezuka K. Dental pulp cells for induced pluripotent stem cell banking. *J Dent Res.* 2010; 89:773–778. [PubMed: 20554890]
257. Miyoshi K, Tsuji D, Kudoh K, Satomura K, Muto T, Itoh K, Noma T. Generation of human induced pluripotent stem cells from oral mucosa. *J Biosci Bioeng.* 2010; 110:345–350. [PubMed: 20547351]
258. Mauritz C, Martens A, Rojas SV, Schnick T, Rathert C, Schecker N, Menke S, Glage S, Zweigerdt R, Haverich A, Martin U, Kutschka I. Induced pluripotent stem cell (iPSC)-derived Flk-1 progenitor cells engraft, differentiate, and improve heart function in a mouse model of acute myocardial infarction. *Eur Heart J.* 2011; 32:2634–2641. [PubMed: 21596799]
259. Ng SL, Narayanan K, Gao S, Wan AC. Lineage restricted progenitors for the repopulation of decellularized heart. *Biomaterials.* 2011; 32:7571–7580. [PubMed: 21783251]
260. Naito H, Melnychenko I, Didie M, Schneiderbanger K, Schubert P, Rosenkranz S, Eschenhagen T, Zimmermann WH. Optimizing engineered heart tissue for therapeutic applications as surrogate heart muscle. *Circulation.* 2006; 114:172–8. [PubMed: 16820649]
261. McMullen JR, Shioi T, Huang WY, Zhang L, Tarnavski O, Bisping E, Schinke M, Kong S, Sherwood MC, Brown J, Riggi L, Kang PM, Izumo S. The insulin-like growth factor 1 receptor

- induces physiological heart growth via the phosphoinositide 3-kinase(p110alpha) pathway. *J Biol Chem*. 2004; 279:4782–4793. [PubMed: 14597618]
262. Sun Y, Jallerat Q, Szymanski JM, Feinberg AW. Conformal nanopatterning of extracellular matrix proteins onto topographically complex surfaces. *Nat Methods*. 2015; 12:134–136. [PubMed: 25506720]
 263. Chang MG, Tung L, Sekar RB, Chang CY, Cysyk J, Dong P, Marban E, Abraham MR. Proarrhythmic potential of mesenchymal stem cell transplantation revealed in an in vitro coculture model. *Circulation*. 2006; 113:1832–1841. [PubMed: 16606790]
 264. Liao B, Christoforou N, Leong KW, Bursac N. Pluripotent stem cell-derived cardiac tissue patch with advanced structure and function. *Biomaterials*. 2011; 32:9180–9187. [PubMed: 21906802]
 265. Narmoneva DA, Vukmirovic R, Davis ME, Kamm RD, Lee RT. Endothelial cells promote cardiac myocyte survival and spatial reorganization: implications for cardiac regeneration. *Circulation*. 2004; 110:962–968. [PubMed: 15302801]
 266. Ieda M, Fu JD, Delgado-Olguin P, Vedantham V, Hayashi Y, Bruneau BG, Srivastava D. Direct reprogramming of fibroblasts into functional cardiomyocytes by defined factors. *Cell*. 2010; 142:375–386. [PubMed: 20691899]
 267. Qian L, Huang Y, Spencer CI, Foley A, Vedantham V, Liu L, Conway SJ, Fu JD, Srivastava D. In vivo reprogramming of murine cardiac fibroblasts into induced cardiomyocytes. *Nature*. 2012; 485:593–598. [PubMed: 22522929]
 268. Parker TG, Packer SE, Schneider MD. Peptide growth factors can provoke “fetal” contractile protein gene expression in rat cardiac myocytes. *J Clin Invest*. 1990; 85:507–514. [PubMed: 1688886]
 269. Kinugawa K, Minobe WA, Wood WM, Ridgway EC, Baxter JD, Ribeiro RC, Tawadrous MF, Lowes BA, Long CS, Bristow MR. Signaling pathways responsible for fetal gene induction in the failing human heart: evidence for altered thyroid hormone receptor gene expression. *Circulation*. 2001; 103:1089–1094. [PubMed: 11222471]
 270. Pasternac A, Cantin M. Atrial natriuretic factor: a ventricular hormone? *J Am Coll Cardiol*. 1990; 15:1446–1448. [PubMed: 2139447]
 271. Tanaka M, Hiroe M, Ito H, Nishikawa T, Adachi S, Aonuma K, Marumo F. Differential localization of atrial natriuretic peptide and skeletal alpha-actin messenger RNAs in left ventricular myocytes of patients with dilated cardiomyopathy. *J Am Coll Cardiol*. 1995; 26:85–92. [PubMed: 7797780]
 272. Lowes BD, Minobe W, Abraham WT, Rizeq MN, Bohlmeier TJ, Quaife RA, Roden RL, Dutcher DL, Robertson AD, Voelkel NF, Badesch DB, Groves BM, Gilbert EM, Bristow MR. Changes in gene expression in the intact human heart. Downregulation of alpha-myosin heavy chain in hypertrophied, failing ventricular myocardium. *J Clin Invest*. 1997; 100:2315–2324. [PubMed: 9410910]
 273. Shioi T, Kang PM, Douglas PS, Hampe J, Yballe CM, Lawitts J, Cantley LC, Izumo S. The conserved phosphoinositide 3-kinase pathway determines heart size in mice. *EMBO J*. 2000; 19:2537–2548. [PubMed: 10835352]
 274. Nadal-Ginard B, Mahdavi V. Molecular basis of cardiac performance. Plasticity of the myocardium generated through protein isoform switches. *J Clin Invest*. 1989; 84:1693–1700. [PubMed: 2687327]
 275. Pieske B, Trost S, Schutt K, Minami K, Just H, Hasenfuss G. Influence of forskolin on the force-frequency behavior in nonfailing and end-stage failing human myocardium. *Basic Res Cardiol*. 1998; 93(Suppl 1):66–75. [PubMed: 9833133]
 276. Hasenfuss G, Schillinger W, Lehnart SE, Preuss M, Pieske B, Maier LS, Prestle J, Minami K, Just H. Relationship between Na⁺-Ca²⁺-exchanger protein levels and diastolic function of failing human myocardium. *Circulation*. 1999; 99:641–648. [PubMed: 9950661]
 277. Zimmermann WH, Melnychenko I, Wasmeier G, Didie M, Naito H, Nixdorff U, Hess A, Budinsky L, Brune K, Michaelis B, Dhein S, Schwoerer A, Ehmke H, Eschenhagen T. Engineered heart tissue grafts improve systolic and diastolic function in infarcted rat hearts. *Nat Med*. 2006; 12:452–458. [PubMed: 16582915]

278. Efimov IR, Nikolski VP, Salama G. Optical imaging of the heart. *Circ Res.* 2004; 95:21–33. [PubMed: 15242982]
279. Duboscq-Bidot L, Xu P, Charron P, Neyroud N, Dilanian G, Millaire A, Bors V, Komajda M, Villard E. Mutations in the Z-band protein myopalladin gene and idiopathic dilated cardiomyopathy. *Cardiovasc Res.* 2008; 77:118–125. [PubMed: 18006477]
280. Severs NJ, Coppen SR, Dupont E, Yeh HI, Ko YS, Matsushita T. Gap junction alterations in human cardiac disease. *Cardiovasc Res.* 2004; 62:368–377. [PubMed: 15094356]
281. Zhang HB, Li RC, Xu M, Xu SM, Lai YS, Wu HD, Xie XJ, Gao W, Ye H, Zhang YY, Meng X, Wang SQ. Ultrastructural uncoupling between T-tubules and sarcoplasmic reticulum in human heart failure. *Cardiovasc Res.* 2013; 98:269–276. [PubMed: 23405000]

Figure 1. Confocal microscopy images of cardiac tissue ultrastructure

(A) An hESC-CM illustrating immature shape and myofibril alignment; magnification = 400X. Reproduced with permission from: Yang et al. (2008) *Nature*. 453(7194):524 [20].

(B) Adult human left ventricle cryosection illustrating highly aligned and organized myofibrils; red = α 2-actinin; scale bar = 10 μ m. Reproduced with permission from: Duboscq-Bidot et al. (2008) *Cardiovasc Res*. 77(1):118 [279].

(C) Confocal reconstruction of an isolated adult human ventricular CM illustrating the localization of gap junctions in intercalated discs; green = connexin-43; red = propidium iodide; scale bar = 50 μ m. Reproduced with permission from: Severs et al. (2004) *Cardiovasc Res*. 62(2):368 [280].

(D) Human ESC-CM EBs (left panel) and hECTs (middle and right panel); scale bar = 20 μ m. Reproduced with permission from: Schaaf et al. (2011) *PLoS One*. 6(10):e26397 [43].

(E) Unstimulated (control, top panels) and electrically-stimulated hECTs (6 Hz, bottom panels); double-headed arrow = suture axis; scale bar = 20 μ m. Reproduced with permission from: Nunes et al. (2013) *Nat Methods*. 10(8):781 [241].

(F) Human ESC-CMs in ECT (left panel) and monolayer (right panel); green = α 2-actinin, red = connexin-43, blue = DAPI. Reproduced with permission from: Zhang et al. (2013) *Biomaterials*. 34(23):5813 [243].

Figure 2. Transmission electron microscopy (TEM) images of cardiac tissue ultrastructure
(A) A schematic of the ultrastructural features of a cardiomyocyte. The grey shading denotes the general banding pattern of the structures as seen by TEM. **(B)** Adult human cardiac tissue indicating uniform sarcomere size, a high degree of alignment, clear H-zones and M-lines, as well as transverse tubules (white arrows). *Insert.* The T-tubule is closely associated with the adjacent SR. Reproduced with permission from: Zhang et al. (2013) *Cardiovasc Res.* 98(2):269 [281]. **(C)** Aggregates of hESC-CMs (*left panel*) and hECTs (*right panel*); red line = sarcomere, black arrow = Z-disc, white arrow = H-zone. Reproduced with permission from: Thavandiran et al (2013) *Proc Natl Acad Sci U S A.* 110(49):E4698 [234]. **(D)** Unstimulated (control, *top panels*) and electrically-stimulated hECTs (6Hz, *bottom panels*) demonstrating sarcomeric structure (white bar = sarcomere, black arrow = Z-disc, white arrow = H zones, m = mitochondria, *left panels*) and desmosomes (white arrows, *right panels*); scale bars = 1 μ m. Reproduced with permission from: Nunes et al. (2013) *Nat Methods.* 10(8):781 [241]. **(E)** Human iPSC-derived EBs (*left panel*) and hECTs (*right panel*); F = myofibril bundle, Z = Z-disc. Reproduced with permission from: Lu et al. (2013) *Nat Commun.* 4:2307 [5].

Figure 3.
Ion current contribution to the phases of the action potential.

Figure 4. A schematic of the intracellular signaling cascades initiated by activation of the β -adrenergic receptor

Activation of the G-protein coupled β -adrenergic receptor induces the conversion of adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP) by adenylyl cyclase (AC) and subsequent activation of protein kinase A (PKA). PKA phosphorylates (P): 1) the $\text{Ca}_v1.2$ channel to increase Ca^{2+} influx, which promotes Ca^{2+} -induced Ca^{2+} release from the sarcoplasmic reticulum (SR) through the ryanodine receptor (RyR); 2) phospholamban (PLB) to increase Ca^{2+} re-uptake into the sarcoplasmic reticulum (SR), which increases the amplitude of the subsequent Ca^{2+} transients and force of contraction; and 3) troponin-I to increase the actin-myosin cross-bridge cycling rate, which increases the rate of contraction. G_s = stimulatory G-protein.

Table 1

A comparison of electrophysiological properties of hPSC-CMs, human fetal CMs and human adult CMs.

	Conductio n Velocity	Action Potentia l Duration	Action Potential Amplitud e	Depolarizatio n Velocity (Vmax)	Resting Membran e Potential	Maximu m Diastolic Potential	Capacitanc e
hPSC-CMs	1–15 cm/s [1, 2]	200–500 ms [1–5]	77–116 mV [3–5]	6–40 V/s [3–5] 176–201 V/s (long-term culture) [6]	–37–71 mV [4–6] –66–70 mV (long-term culture) [6]	–57–75 mV [3]	10–25 pF [3, 7]
Human Fetal CMs	40–70 cm/s (whole hearts) [8]	324–416 ms [4]	20–27 mV [4]	5–13 V/s [4]	–37–40 mV [4] –82mV (whole hearts) [8]	–85–86 mV [9]	16–25 pF [10]
Human Adult CMs	30–100 cm/s [11]	228–259 ms [9]	102–110 mV [9]	254–303 V/s [9]	~ –90 mV [12]	–82–90 mV [12]	~200pF [12–14]

[1] P.W. Burridge, S. Thompson, M.A. Millrod, S. Weinberg, X. Yuan, A. Peters, V. Mahairaki, V.E. Koliatsos, L. Tung, E.T. Zambidis, A universal system for highly efficient cardiac differentiation of human induced pluripotent stem cells that eliminates interline variability, PLoS One 6 (2011) e18293.

[2] S.A. Thompson, P.W. Burridge, E.A. Lipke, M. Shambloott, E.T. Zambidis, L. Tung, Engraftment of human embryonic stem cell derived cardiomyocytes improves conduction in an arrhythmogenic in vitro model, J. Mol. Cell. Cardiol. 53 (2012) 15–23.

[3] M.X. Doss, J.M. Di Diego, R.J. Goodrow, Y. Wu, J.M. Cordeiro, V.V. Nesterenko, H. Barajas-Martinez, D. Hu, J. Urrutia, M. Desai, J.A. Treat, A. Sachinidis, C. Antzelevitch, Maximum diastolic potential of human induced pluripotent stem cell-derived cardiomyocytes depends critically on I(Kr), PLoS One 7 (2012) e40288.

[4] C. Mummery, D. Ward-van Oostwaard, P. Doevendans, R. Spijker, S. van den Brink, R. Hassink, M. van der Heyden, T. Ophhof, M. Pera, A.B. de la Riviere, R. Passier, L. Tertoolen, Differentiation of human embryonic stem cells to cardiomyocytes: role of coculture with visceral endoderm-like cells, Circulation 107 (2003) 2733–2740.

[5] M.C. Ribeiro, L.G. Tertoolena, J.A. Guadix, M. Bellina, G. Kosmidisa, C. D’Anielloa, J. Monshouwer-Klootsa, M.J. Goumans, Y.L. Wang, A.W. Feinberg, C.L. Mummery, R. Passiera, Functional maturation of human pluripotent stem cell derived cardiomyocytes *in vitro*—Correlation between contraction force and electrophysiology, Biomaterials 51 (2015) 138–150.

[6] S.D. Lundy, W.Z. Zhu, M. Regnier, M.A. Laflamme, Structural and functional maturation of cardiomyocytes derived from human pluripotent stem cells, Stem Cells Dev. 22 (2013) 1991–2002.

[7] X. Sheng, M. Reppel, F. Nguemo, F.I. Mohammad, A. Kuzmenkin, J. Hescheler, K. Pfannkuche, Human pluripotent stem cell-derived cardiomyocytes: response to TTX and lidocain reveals strong cell to cell variability, PLoS One 7 (2012) e45963.

[8] G. Gennser, E. Nilsson, Excitation and impulse conduction in the human fetal heart, Acta Physiol. Scand. 79 (1970) 305–320.

[9] I. Koncz, T. Szel, M. Bitay, E. Cerbai, K. Jaeger, F. Fulop, N. Jost, L. Virag, P. Orvos, L. Talosi, A. Kristof, I. Baczko, J.G. Papp, A. Varro, Electrophysiological effects of ivabradine in dog and human cardiac preparations; potential antiarrhythmic actions, Eur. J. Pharmacol. 668 (2011) 419–426.

[10] W.Z. Zhu, L.F. Santana, M.A. Laflamme, Local control of excitation-contraction coupling in human embryonic stem cell-derived cardiomyocytes, PLoS One 4 (2009) e5407.

[11] X. Yang, L. Pabon, C.E. Murry, Engineering adolescence: maturation of human pluripotent stem cell-derived cardiomyocytes, Circ. Res. 114 (2014) 511–523.

[12] E. Drouin, F. Charpentier, C. Gauthier, K. Laurent, H. Le Marec, Electrophysiologic characteristics of cells spanning the left ventricular wall of human heart: evidence for presence of M cells, J. Am. Coll. Cardiol. 26 (1995) 185–192.

[13] W.Z. Zhu, Y. Xie, K.W. Moyes, J.D. Gold, B. Askari, M.A. Laflamme, Neuregulin/ErbB signaling regulates cardiac subtype specification in differentiating human embryonic stem cells, Circ. Res. 107 (2010) 776–786.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

114] S. Polak, K. Fijorek, Inter-individual variability in the pre-clinical drug cardiotoxic safety assessment--analysis of the age-cardiomyocytes electric capacitance dependence, J. Cardiovasc. Transl. Res. 5 (2012) 321–332.

Table 2

A comparison of the electrical properties of hECTs.

Ref	Electrical Measurement Conditions	Conduction Velocity	Action Potential Duration	Action Potential Amplitude	Depolarization Velocity (Vmax)	Other Measurements
Kensah <i>et al</i> [1]	Microelectrode Array: hECTs; spontaneous beating	4.9 cm/s				
Mihic <i>et al</i> [2]	Optical Mapping: calcium-sensitive dye; spontaneous beating		430–570 ms (Ca ²⁺ cycle durations)			
Schaaf <i>et al</i> [3]	Patch clamp: spontaneous beating		318–364 ms 816–958 ms	62–70 mV (APD<500ms) 82–88 mV (APD>500ms)	5–6 V/s (APD<500ms) 9–12 V/s (APD>500ms)	MDP = –47–51 mV
Turnbull <i>et al</i> [4]	Optical mapping: voltage-sensitive dye; 2Hz; field stimulation		143–225 ms			
Thavandiran <i>et al</i> [5]	Optical Mapping: voltage-sensitive dye	17–33cm/s				
Nunes <i>et al</i> [6]	Ca ²⁺ transients: single CMs; spontaneous beating Optical Mapping: voltage-sensitive dye; hECT; point stimulation Patch clamp: 1Hz	~11.5–18.5 cm/s (optical mapping)	100–150 ms (patch clamp)	~70 mV (patch clamp)	~125V/s (patch clamp)	RMP ≈ –100mV (patch clamp) Capacitance = 18–21 pF (patch clamp)
Zhang <i>et al</i> [7]	Optical Mapping: voltage-sensitive dye; 0.5Hz; point stimulation	25.1 cm/s	308–368 ms			

[1] G. Kensah, I. Grnh, J. Viering, H. Schumann, J. Dahlmann, H. Meyer, D. Skvorc, A. Bar, P. Akhyari, A. Heisterkamp, A. Haverich, U. Martin, A novel miniaturized multimodal bioreactor for continuous in situ assessment of bioartificial cardiac tissue during stimulation and maturation, *Tissue Eng. Part C. Methods* 17 (2011) 463–473.

[2] A. Mihic, J. Li, Y. Miyagi, M. Gagliardi, S.H. Li, J. Zu, R.D. Weisel, G. Keller, R.K. Li, The effect of cyclic stretch on maturation and 3D tissue formation of human embryonic stem cell-derived cardiomyocytes, *Biomaterials* 35 (2014) 2798–2808.

[3] S. Schaaf, A. Shibamiya, M. Mewe, A. Eder, A. Stohr, M.N. Hirt, T. Rau, W.H. Zimmermann, L. Conradi, T. Eschenhagen, A. Hansen, Human engineered heart tissue as a versatile tool in basic research and preclinical toxicology, *PLoS One* 6 (2011) e26397.

[4] I.C. Turnbull, I. Karakikes, G.W. Serrao, P. Backeris, J.J. Lee, C. Xie, G. Senyei, R.E. Gordon, R.A. Li, F.G. Akar, R.J. Hajjar, J.S. Hulot, K.D. Costa, Advancing functional engineered cardiac tissues toward a preclinical model of human myocardium, *FASEB J.* 28 (2014) 644–654.

[5] N. Thavandiran, N. Dubois, A. Mikryukov, S. Masse, B. Beca, C.A. Simmons, V.S. Deshpande, J.P. McGarry, C.S. Chen, K. Nanthakumar, G.M. Keller, M. Radisic, P.W. Zandstra, Design and formulation of functional pluripotent stem cell-derived cardiac microtissues, *Proc. Natl. Acad. Sci. U. S. A.* 110 (2013) E4698–707.

[6] S.S. Nunes, J.W. Miklas, J. Liu, R. Aschar-Sobbi, Y. Xiao, B. Zhang, J. Jiang, S. Masse, M. Gagliardi, A. Hsieh, N. Thavandiran, M.A. Laflamme, K. Nanthakumar, G.J. Gross, P.H. Backx, G. Keller, M. Radisic, Biowire: a platform for maturation of human pluripotent stem cell-derived cardiomyocytes, *Nat. Methods* 10 (2013) 781–787.

[7] D. Zhang, I.Y. Shadrin, J. Lam, H.Q. Xian, H.R. Snodgrass, N. Bursac, Tissue-engineered cardiac patch for advanced functional maturation of human ESC-derived cardiomyocytes, *Biomaterials* 34 (2013) 5813–5820.

Table 3

A comparison of the contractile properties of hECTs.

Ref	Force Measurement Conditions	Force-Frequency Relationship (FFR) & Frank-Starling Curve (FSC)	Contractile Force	Contractile Stress	Excitation Threshold (ET) & Maximum Capture Rate (MCR)	Spontaneous Beat Rate	Maximum Rate of Force Increase (+dF/dt) & Decrease (-dF/dt)	Time to Peak Force (TPF) & Time to 90% Relaxation (RT ₉₀)
Tulloch <i>et al</i> [1]	Force Transducer; spontaneous	Positive FSC	0.02 mN	~0.1 mN/m m ²		60 bpm		
Kensah <i>et al</i> [2]	Custom-Made Reactor; field stimulation (25V, 10ms)	Positive FSC	1.2–1.6 mN	4.4 mN/m m ²		51–58 bpm		TPF = 47–59 ms RT ₉₀ = 63–69 ms
Mihic <i>et al</i> [3]	Optical Mapping; calcium-sensitive dye					63–78 Ca ²⁺ transient cycles/min		
Streckfuss-Böncke <i>et al</i> [4]	Custom-Made Reactor; field stimulation (1.5Hz, 5ms, V = ET +10%)	Positive FSC	0.2 mN					
Schaaf <i>et al</i> [5]	Post-Deflection; spontaneous		0.05–0.07 mN	~0.1 mN/m m ²		40–70 bpm	+dF/dt = 10 mN/s -dF/dt = 6 mN/s	TPF = 200 ms RT ₉₀ = 300 ms
Turnbull <i>et al</i> [6]	Post-Deflection; field stimulation (2Hz, 5ms) Physiological Muscle Bath; field stimulation	Negative FFR Positive FSC	0.6 mN	0.5–0.6 mN/m m ² (muscle bath, 1Hz) 0.03–0.2 mN/m m ² (post-deflection, 1Hz)	ET = 0.4–2.2 V/cm (post-deflection) MCR = 3Hz (post-deflection)	42–100 bpm (post-deflection)	+dF/dt = 7–8 mN/mm ² /s -dF/dt = 4–6 mN/mm ² /s (post-deflection)	TPF = 80–96 ms RT ₉₀ = 106–127 ms (post-deflection)
Thavandiran <i>et al</i> [7]	Post-Deflection; field stimulation (2ms, 1–2Hz or 12V)				ET = 1.8–2.4 V/cm MCR = 6.1–6.5 Hz	30–60 bpm		
Nunes <i>et al</i> [8]	Optical Mapping; voltage-sensitive dye				ET ≈ 0.8–2.3 V/cm (field stimulation) MCR = 2.8–4.8 Hz (point stimulation) MCR = 5.2 Hz (field stimulation)			

Ref	Force Measurement Conditions	Force-Frequency Relationship (FFR) & Frank-Starling Curve (FSC)	Contractile Force	Contractile Stress	Excitation Threshold (ET) & Maximum Capture Rate (MCR)	Maximum Rate of Force Increase (+dF/dt) & Force Decrease (-dF/dt)	Time to Peak Force (TPF) & Time to 90% Relaxation (RT ₉₀)
Hirt <i>et al</i> [9]	Post-Deflection; spontaneous		0.08 mN	~2.2 mN/m ²			74–90 bpm
Zhang <i>et al</i> [10]	Custom-Made Reactor: field stimulation (1Hz)	Positive FSC	1.9–4.1 mN	7.3–16.3 mN/m ²	MCR = 2.4–2.8 Hz (optical mapping, point stimulation)		
Lu <i>et al</i> [11]	Force Transducer: field stimulation (1Hz, 5ms, V = ET+20%)	Negative FFR	0.2 mN				40–50 bpm

[1] N.L. Tulloch, V. Muskheli, M.V. Razumova, F.S. Korte, M. Regnier, K.D. Hauch, L. Pabon, H. Reinecke, C.E. Murry, Growth of engineered human myocardium with mechanical loading and vascular coculture, *Circ. Res.* 109 (2011) 47–59.

[2] G. Kensah, I. Gruh, J. Viering, H. Schumann, J. Dahlmann, H. Meyer, D. Skvorc, A. Bar, P. Akhyari, A. Heisterkamp, A. Haverich, U. Martin, A novel miniaturized multimodal bioreactor for continuous in situ assessment of bioartificial cardiac tissue during stimulation and maturation, *Tissue Eng. Part C. Methods* 17 (2011) 463–473.

[3] A. Mihic, J. Li, Y. Miyagi, M. Gagliardi, S.H. Li, J. Zu, R.D. Weisel, G. Keller, R.K. Li, The effect of cyclic stretch on maturation and 3D tissue formation of human embryonic stem cell-derived cardiomyocytes, *Biomaterials* 35 (2014) 2798–2808.

[4] K. Streckfuss-Bomeke, F. Wolf, A. Azizian, M. Stauske, M. Tiburecy, S. Wagner, D. Hubscher, R. Dressel, S. Chen, J. Jende, G. Wulf, V. Lorenz, M.P. Schon, L.S. Maier, W.H. Zimmermann, G. Hasenfuss, K. Guan, Comparative study of human-induced pluripotent stem cells derived from bone marrow cells, hair keratinocytes, and skin fibroblasts, *Eur. Heart J.* 34 (2013) 2618–2629.

[5] S. Schaaf, A. Shibamiya, M. Mewe, A. Eder, A. Stohr, M.N. Hirt, T. Rau, W.H. Zimmermann, L. Conradi, T. Eschenhagen, A. Hansen, Human engineered heart tissue as a versatile tool in basic research and preclinical toxicology, *PLoS One* 6 (2011) e26397.

[6] L.C. Turnbull, I. Karakikes, G.W. Serrao, P. Backeris, J.J. Lee, C. Xie, G. Senyei, R.E. Gordon, R.A. Li, F.G. Akar, R.J. Hajjar, J.S. Hulot, K.D. Costa, Advancing functional engineered cardiac tissues toward a preclinical model of human myocardium, *FASEB J.* 28 (2014) 644–654.

[7] N. Thavandiran, N. Dubois, A. Mikryukov, S. Masse, B. Beca, C.A. Simmons, V.S. Deshpande, J.P. McGarry, C.S. Chen, K. Nanthakumar, G.M. Keller, M. Radisic, P.W. Zandstra, Design and formulation of functional pluripotent stem cell-derived cardiac microtissues, *Proc. Natl. Acad. Sci. U. S. A.* 110 (2013) E4698–707.

[8] S.S. Nunes, J.W. Miklas, J. Liu, R. Aschar-Sobbi, Y. Xiao, B. Zhang, J. Jiang, S. Masse, M. Gagliardi, A. Hsieh, N. Thavandiran, M.A. Laflamme, K. Nanthakumar, G.J. Gross, P.H. Backx, G. Keller, M. Radisic, Biowire: a platform for maturation of human pluripotent stem cell-derived cardiomyocytes, *Nat. Methods* 10 (2013) 781–787.

[9] M.N. Hirt, J. Boedinghaus, A. Mitchell, S. Schaaf, C. Borchers, C. Muller, H. Schulz, N. Hubner, J. Stenzig, A. Stoeher, C. Neuber, A. Eder, P.K. Luther, A. Hansen, T. Eschenhagen, Functional improvement and maturation of rat and human engineered heart tissue by chronic electrical stimulation, *J. Mol. Cell. Cardiol.* 74 (2014) 151–161.

[10] D. Zhang, I.Y. Shadrin, J. Lam, H.Q. Xian, H.R. Snodgrass, N. Bursac, Tissue-engineered cardiac patch for advanced functional maturation of human ESC-derived cardiomyocytes, *Biomaterials* 34 (2013) 5813–5820.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

///T.Y. Lu, B. Lin, J. Kim, M. Sullivan, K. Tobita, G. Salama, L. Yang. Repopulation of decellularized mouse heart with human induced pluripotent stem cell-derived cardiovascular progenitor cells, Nat. Commun. 4 (2013) 2307.

Table 4

A comparison of the pharmacological responses of hECTs.

Reference	Measurement Conditions	Catecholamines	Calcium	L-type Ca^{2+} channel blocker	Other
Kensah <i>et al</i> [1]	Microelectrode Array: hECT; spontaneous beating Custom-Made Reactor: field stimulation (25V, 10ms)	Isoproterenol: 0.1 μM $\uparrow$ rate (MEA) $\uparrow$ rate, $\uparrow$ force (custom-made reactor)			Carbachol: 10 μM added after isoproterenol $\downarrow$ rate (custom-made reactor)
Streckfuss-Bömeke <i>et al</i> [2]	Custom-Made Reactor: field stimulation (1.5Hz, 5ms, V = ET+10%)	Isoproterenol: 1 μM $\uparrow$ force, $\uparrow$ relaxation velocity (custom-made reactor)	0.2–2.8mM $\uparrow$ force $\text{EC}_{50} = 0.4\text{mM}$ (custom-made reactor)		
Schaaf <i>et al</i> [3]	Post-deflection: spontaneous Patch clamping: spontaneous	Isoproterenol: 100nM $\uparrow$ rate (post-deflection)	0.2–3.0mM $\uparrow$ force $\text{EC}_{50} = 0.8\text{--}1.0\text{mM}$ (post-deflection)	Nisoldipine: 1 μM $\downarrow$ amplitude (patch clamp)	Carbachol: 10 μM after isoproterenol $\downarrow$ rate (post-deflection) E-4031: 300nM $\uparrow$ APD (patch clamp) 1–30nM $\downarrow$ force, $\downarrow$ Vmax, $\uparrow$ beat-to-beat variability (post-deflection) Tetrodotoxin: 3 μM $\downarrow$ Vmax, $\uparrow$ APD, $\uparrow$ DI (patch clamp)
Tumbull <i>et al</i> [4]	Post-Deflection: spontaneous Physiological Muscle Bath: field stimulation (2Hz, 5ms)	Isoproterenol: 1nM–1 μM $\uparrow$ rate (post-deflection) 1nM–10 μM $\uparrow$ force $\text{EC}_{50} = 750\text{nM}$ (muscle bath)	0.5–2.5mM $\uparrow$ force $\text{EC}_{50} = 1.8\text{mM}$ (muscle bath)	Verapamil: 1nM–10 μM $\downarrow$ force $\text{IC}_{50} = 0.61 \mu\text{M}$ (muscle bath)	
Thavandiran <i>et al</i> [5]	Optical Mapping: voltage sensitive dye; point stimulation (1Hz) Optical Mapping: calcium-sensitive dye	Epinephrine: 500nM $\uparrow$ rate (AP propagation)		Verapamil: 500nM $\downarrow$ amplitude (Ca^{2+} transient)	Lidocaine: 8.5 μM after epinephrine $\downarrow$ rate (AP propagation)
Nunes <i>et al</i> [6]	Optical Mapping: calcium-sensitive dye; spontaneous			Verapamil: 1mM no Ca^{2+} transients Nifedipine: 10 μM no Ca^{2+} transients	Caffeine: 5mM $\uparrow$ Ca^{2+} amplitude Thapsigargin: 2 μM no Ca^{2+} transients
Zhang <i>et al</i> [7]	Custom-Made Reactor: field stimulation (1Hz)	Isoproterenol: 0.1nM–1 μM $\uparrow$ rate, $\uparrow$ force $\text{EC}_{50} = 95.1 \text{ nM}$			
Lu <i>et al</i> [8]	Optical Mapping: calcium-sensitive dye (spontaneous) Force Transducer: field stimulation (1.5Hz, 5ms, V = ET+20%)	Isoproterenol: 1nM–5 μM $\uparrow$ rate = 10- EC_{50} 100nM (Ca^{2+} transient)	5nM $\uparrow$ force (force transducer)		E-4031: 1 μM $\downarrow$ amplitude, pulsus alternans (Ca^{2+} transient)
hPSC-CMs		Isoproterenol: $\text{EC}_{50} = 12.9\text{nM}$ [9]	$\text{EC}_{50} = 0.4\text{--}1.8\text{mM}$ [2, 4]	Nifedipine: $\text{IC}_{50} = 39\text{nM}$ [10]	E-4031: $\text{IC}_{50} = 17\text{nM}$ [10, 11]
Human Adult		Isoproterenol: $\text{EC}_{50} = 11\text{--}80\text{nM}$ [12–15]	$\text{EC}_{50} = 3.0\text{mM}$ [16]	Verapamil: $\text{IC}_{50} = 143 \text{ nM}$ (hERG) [11, 17]	Carbachol: $\text{EC}_{50} = 140\text{nM}$ [23] E-4031: $\text{IC}_{50} = 7\text{--}32\text{nM}$ [11, 20] Tetrodotoxin: $\text{IC}_{50} = 1 \mu\text{M}$ [27, 28]

Reference	Measurement Conditions	Catecholamines	Calcium	L-type Ca^{2+} channel blocker	Other
[11]	G. Kensah, I. Gruh, J. Vierung, H. Schumann, J. Dahlmann, H. Meyer, D. Skvorc, A. Bar, P. Akhyari, A. Heisterkamp, A. Haverich, U. Martin, A novel miniaturized multimodal bioreactor for continuous in situ assessment of bioartificial cardiac tissue during stimulation and maturation, <i>Tissue Eng. Part C: Methods</i> 17 (2011) 463–473.			$\text{IC}_{50} = 0.24\text{--}4.3\mu\text{M}$ (Ca, 1.2) [18–20]	Lidocaine: $\text{IC}_{50} = 34\text{--}38\mu\text{M}$ [29]
[12]	K. Streckfuss-Bomeke, F. Wolf, A. Azizian, M. Stauske, M. Tiburecy, S. Wagner, D. Hubscher, R. Dressel, S. Chen, J. Jende, G. Wulf, V. Lorenz, M.P. Schon, L.S. Maier, W.H. Zimmermann, G. Hasenfuss, K. Guan, Comparative study of human-induced pluripotent stem cells derived from bone marrow cells, hair keratinocytes, and skin fibroblasts, <i>Eur. Heart J.</i> 34 (2013) 2618–2629.			$\text{IC}_{50} = 123.0\text{nM}$ (inotropic) [21]	
[13]	S. Schaaf, A. Shibamiya, M. Mewe, A. Eder, A. Stohr, M.N. Hirt, T. Rau, W.H. Zimmermann, L. Conradi, T. Eschenhagen, A. Hansen, Human engineered heart tissue as a versatile tool in basic research and preclinical toxicology, <i>PLoS One</i> 6 (2011) e26397.			Nifedipine: $\text{IC}_{50} = 16\text{--}24\text{ nM}$ [19]	
[14]	J.C. Tumbull, I. Karakikes, G.W. Serrao, P. Backeris, J.J. Lee, C. Xie, G. Senyei, R.E. Gordon, R.A. Li, F.G. Akar, R.J. Hajjar, J.S. Hulot, K.D. Costa, Advancing functional engineered cardiac tissues toward a preclinical model of human myocardium, <i>FASEB J.</i> 28 (2014) 644–654.			Nisoldipine: $\text{IC}_{50} = 67\text{--}81\text{ nM}$ [22]	

[11] G. Kensah, I. Gruh, J. Vierung, H. Schumann, J. Dahlmann, H. Meyer, D. Skvorc, A. Bar, P. Akhyari, A. Heisterkamp, A. Haverich, U. Martin, A novel miniaturized multimodal bioreactor for continuous in situ assessment of bioartificial cardiac tissue during stimulation and maturation, *Tissue Eng. Part C: Methods* 17 (2011) 463–473.

[12] K. Streckfuss-Bomeke, F. Wolf, A. Azizian, M. Stauske, M. Tiburecy, S. Wagner, D. Hubscher, R. Dressel, S. Chen, J. Jende, G. Wulf, V. Lorenz, M.P. Schon, L.S. Maier, W.H. Zimmermann, G. Hasenfuss, K. Guan, Comparative study of human-induced pluripotent stem cells derived from bone marrow cells, hair keratinocytes, and skin fibroblasts, *Eur. Heart J.* 34 (2013) 2618–2629.

[13] S. Schaaf, A. Shibamiya, M. Mewe, A. Eder, A. Stohr, M.N. Hirt, T. Rau, W.H. Zimmermann, L. Conradi, T. Eschenhagen, A. Hansen, Human engineered heart tissue as a versatile tool in basic research and preclinical toxicology, *PLoS One* 6 (2011) e26397.

[14] J.C. Tumbull, I. Karakikes, G.W. Serrao, P. Backeris, J.J. Lee, C. Xie, G. Senyei, R.E. Gordon, R.A. Li, F.G. Akar, R.J. Hajjar, J.S. Hulot, K.D. Costa, Advancing functional engineered cardiac tissues toward a preclinical model of human myocardium, *FASEB J.* 28 (2014) 644–654.

[15] N. Thavandiran, N. Dubois, A. Mikryukov, S. Masse, B. Beca, C.A. Simmons, V.S. Deshpande, J.P. McGarry, C.S. Chen, K. Nanthakumar, G.M. Keller, M. Radisic, P.W. Zandstra, Design and formulation of functional pluripotent stem cell-derived cardiac microtissues, *Proc. Natl. Acad. Sci. U. S. A.* 110 (2013) E4698–707.

[16] S.S. Nunes, J.W. Miklas, J. Liu, R. Aschar-Sobbi, Y. Xiao, B. Zhang, J. Jiang, S. Masse, M. Gagliardi, A. Hsieh, N. Thavandiran, M.A. Laflamme, K. Nanthakumar, G.J. Gross, P.H. Backx, G. Keller, M. Radisic, Biowire: a platform for maturation of human pluripotent stem cell-derived cardiomyocytes, *Nat. Methods* 10 (2013) 781–787.

[17] D. Zhang, I.Y. Shadrin, J. Lam, H.Q. Xian, H.R. Snodgrass, N. Bursac, Tissue-engineered cardiac patch for advanced functional maturation of human ESC-derived cardiomyocytes, *Biomaterials* 34 (2013) 5813–5820.

[18] T.Y. Lu, B. Lin, J. Kim, M. Sullivan, K. Tobita, G. Salama, L. Yang, Repopulation of decellularized mouse heart with human induced pluripotent stem cell-derived cardiovascular progenitor cells, *Nat. Commun.* 4 (2013) 2307.

[19] M. Brito-Martins, S.E. Harding, N.N. Ali, Beta(1)- and Beta(2)-Adrenoceptor Responses in Cardiomyocytes Derived from Human Embryonic Stem Cells: Comparison with Failing and Non-Failing Adult Human Heart, *Br. J. Pharmacol.* 153 (2008) 751–759.

[10] J.K. Gibson, Y. Yue, J. Bronson, C. Palmer, R. Numann, Human stem cell-derived cardiomyocytes detect drug-mediated changes in action potentials and ion currents, *J. Pharmacol. Toxicol. Methods* 70 (2014) 255–267.

[11] K. Harris, M. Aylott, Y. Cui, J.B. Louttit, N.C. McMahon, A. Sridhar, Comparison of electrophysiological data from human-induced pluripotent stem cell-derived cardiomyocytes to functional preclinical safety assays, *Toxicol. Sci.* 134 (2013) 412–426.

[12] M. Flesch, R.H. Schwinger, F. Schiffer, K. Frank, M. Sudkamp, F. Kuhn-Regnier, G. Arnold, M. Bohm, Evidence for functional relevance of an enhanced expression of the Na^{+} - Ca^{2+} exchanger in failing human myocardium, *Circulation* 94 (1996) 992–1002.

- [13] C. Holubarsch, R. Schneider, B. Pieske, T. Ruf, G. Hasenfuss, G. Fraedrich, H. Posival, H. Just, Positive and negative inotropic effects of DL-sotalol and D-sotalol in failing and nonfailing human myocardium under physiological experimental conditions, *Circulation* 92 (1995) 2904–2910.
- [14] M. Bohm, I. Morano, B. Pieske, J.C. Ruegg, M. Wankel, R. Zimmermann, E. Erdmann, Contribution of cAMP-phosphodiesterase inhibition and sensitization of the contractile proteins for calcium to the inotropic effect of pimobendan in the failing human myocardium, *Circ. Res.* 68 (1991) 689–701.
- [15] M. White, R. Roden, W. Minobe, M.F. Khan, P. Larrabee, M. Wollmering, J.D. Port, F. Anderson, D. Campbell, A.M. Feldman, Age-related changes in beta-adrenergic neuroeffector systems in the human heart, *Circulation* 90 (1994) 1225–1238.
- [16] B.S. Cain, D.R. Meldrum, X. Meng, B.D. Shames, A. Banerjee, A.H. Harken, Calcium preconditioning in human myocardium, *Ann. Thorac. Surg.* 65 (1998) 1065–1070.
- [17] S. Zhang, Z. Zhou, Q. Gong, J.C. Makielski, C.T. January, Mechanism of block and identification of the verapamil binding domain to HERG potassium channels, *Circ. Res.* 84 (1999) 989–998.
- [18] B.S. Freeze, M.M. McNulty, D.A. Hanck, State-dependent verapamil block of the cloned human Ca(v)3.1 T-type Ca(2+) channel, *Mol. Pharmacol.* 70 (2006) 718–726.
- [19] Y.A. Kuryshv, A.M. Brown, E. Duzic, G.E. Kirsch, Evaluating state dependence and subtype selectivity of calcium channel modulators in automated electrophysiology assays, *Assay Drug Dev. Technol.* 12 (2014) 110–119.
- [20] K. Yamazaki, T. Hihara, H. Kato, T. Fukushima, K. Fukushima, T. Taniguchi, T. Yoshinaga, N. Miyamoto, M. Ito, K. Sawada, Beat-to-Beat Variability in Field Potential Duration in Human Embryonic Stem Cell-Derived Cardiomyocyte Clusters for Assessment of Arrhythmogenic Risk, and a Case Study of Its Application, *Pharmacology and Pharmacy* 5 (2014) 117–128.
- [21] D. Sarsero, T. Fujiwara, P. Molenaar, J.A. Angus, Human vascular to cardiac tissue selectivity of L- and T-type calcium channel antagonists, *Br. J. Pharmacol.* 125 (1998) 109–119.
- [22] I. Splawski, K.W. Timothy, L.M. Sharpe, N. Decher, P. Kumar, R. Bloise, C. Napolitano, P.J. Schwartz, R.M. Joseph, K. Condouris, H. Tager-Flusberg, S.G. Priori, M.C. Sanguinetti, M.T. Keating, Ca(V)1.2 calcium channel dysfunction causes a multisystem disorder including arrhythmia and autism, *Cell* 119 (2004) 19–31.
- [23] J. Koglin, M. Bohm, W. von Scheidt, A. Stablein, E. Erdmann, Antiadrenergic effect of carbachol but not of adenosine on contractility in the intact human ventricle in vivo, *J. Am. Coll. Cardiol.* 23 (1994) 678–683.
- [24] W.A. Catterall, A.L. Goldin, S.G. Waxman, International Union of Pharmacology. XLVII. Nomenclature and structure-function relationships of voltage-gated sodium channels, *Pharmacol. Rev.* 57 (2005) 397–409.
- [25] A.L. Goldin, Resurgence of sodium channel research, *Annu. Rev. Physiol.* 63 (2001) 871–894.
- [26] P.B. Bennett, C. Valenzuela, L.Q. Chen, R.G. Kallen, On the molecular nature of the lidocaine receptor of cardiac Na+ channels. Modification of block by alterations in the alpha-subunit III–IV interdomain, *Circ. Res.* 77 (1995) 584–592.

Table 5

A comparison of the pharmacological responses of human adult and fetal CMs/myocardium and hPSC-CMs.

Drug Name	Target	Effect in Human Adult CMs/Myocardium	Effect in Human Fetal CMs/Myocardium	Effect in hPSC-CMs
Epinephrine (EPI) Norepinephrine (NOR) Isoproterenol (ISO)	β -adrenergic receptor agonist	<ul style="list-style-type: none"> Positive inotropic, chronotropic and lusitropic effects [1, 2] Right-shifted force-Ca^{2+} relationship (3μM ISO; RV trabecular skinned and intact muscle) [3] EC_{50} = 11–80nM (ISO; inotropic; LV muscle strips) [4, 8] EC_{50} = 1.21–2.43 μM (NOR; inotropic; LV papillary muscle strips) [8] 	<ul style="list-style-type: none"> Positive isotropic effect (NOR, ISO; 12–22 week fetal atria) [9] Gestation-dependent $\uparrow$ in inotropic effects (0.1μM ISO; 20% at week 12 to 150% at week 22; fetal atria) [9] $\uparrow$peak Ca^{2+} transient and Ca^{2+} transient decay (ISO; monolayer) [10] Positive chronotropic effect (EPI, NOR, ISO; 5–24 week fetal heart) [11] 	<ul style="list-style-type: none"> Positive chronotropic effect (ISO; hESC-CMs) [12–14] EC_{50} = 12.9nM (ISO; chronotropic; hESC EBs) [15]
Carbachol	Cholinergic agonist	<ul style="list-style-type: none"> Negative inotropic, chronotropic and dromotropic effects [16–18] EC_{50} = 140nM (inotropic; LV papillary muscle strips) [19] 	<ul style="list-style-type: none"> Negative inotropic effect (0.1–10μM; 12–20 week fetal hearts) [20] No chronotropic effect (100μM; 16–17 week ventricular CMs) [14] Gestation-dependent $\downarrow$ in repolarization time (10μM, no effect in 12–13 week hearts; 10μM, 21% $\downarrow$ in 14 week hearts; 1–10μM, 26–50% $\downarrow$ in 16–20 week hearts) 	<ul style="list-style-type: none"> Negative chronotropic (hESC-CMs) [14]
Verapamil	$\text{K}_V11.1$ (hERG) & $\text{Ca}_V1.2$ channel antagonist	<ul style="list-style-type: none"> Negative inotropic, chronotropic and dromotropic IC_{50} = 143nM ($\text{K}_V11.1$; 	<ul style="list-style-type: none"> Inhibition of AP propagation (5μM; 16–17 week ventricular CMs) [14] 	<ul style="list-style-type: none"> Inhibition of AP propagation (5μM; hESC-CMs) [14] Negative inotropic, positive chronotropic

Drug Name	Target	Effect in Human Adult CMs/Myocardium	Effect in Human Fetal CMs/Myocardium	Effect in hPSC-CMs
		<ul style="list-style-type: none"> transfected cell line) [21, 22] • $IC_{50} = 0.24\text{--}4.3\mu\text{M}$ ($Ca_v1.2$; transfected cell line) [23, 25] • $IC_{50} = 0.32\text{--}1.21\mu\text{M}$ (inotropic; LV papillary muscle strips) [26] • $IC_{50} = 89\text{--}170\text{nM}$ (inotropic; RA trabeculae muscle strips) [27] 		<ul style="list-style-type: none"> and $\downarrow$FPD ($0.01\text{--}0.3\mu\text{M}$; hiPSC-CM monolayer) [22, 28] • Negative chronotropic and $\downarrow$FPD ($1\text{--}5\mu\text{M}$; hiPSC-CM clusters) [29] • $IC_{50} = 62\text{--}80\text{nM}$ (L-type Ca^{2+} channel; hiPSC-CM monolayer) [30] • $IC_{50} = 73\text{--}120\text{nM}$ (L-type Ca^{2+} channel; hESC-CM monolayer) [30]
Nifedipine	L-type Ca^{2+} channel antagonist	<ul style="list-style-type: none"> • Negative inotropic effect [26, 27, 31] • $IC_{50} = 16\text{--}24\text{nM}$ ($Ca_v1.2$; transfected cell line) [24] • $IC_{50} = 50\text{--}200\text{nM}$ (inotropic; ventricular muscle) [26, 31] • $IC_{50} = 76\text{--}166\text{nM}$ (inotropic; RA trabeculae muscle strips) [27] 	<ul style="list-style-type: none"> • Negative inotropic effect ($10\text{--}20\mu\text{M}$; 7–9 week beating clusters) [32] • Blocked L-type Ca^{2+} current ($10\mu\text{M}$; 20 week CMs) [33] 	<ul style="list-style-type: none"> • Negative inotropic, positive chronotropic and $\downarrow$FPD ($0.003\text{--}1\mu\text{M}$; hiPSC-CM monolayer) [22, 28] • $\downarrow$APD ($1\mu\text{M}$; hESC-CMs, hiPSC-CMs) [34] • Blocked AP propagation ($10\mu\text{M}$; hESC-CMs, hiPSC-CMs) [34] • $IC_{50} = 39\text{nM}$ (Ca^{2+} current; hiPSC-CMs) [35] • $IC_{50} = 2\text{--}4\text{nM}$ (L-type Ca^{2+} channel; hiPSC-CM monolayer) [30] • $IC_{50} = 5\text{--}7\text{nM}$ (L-type Ca^{2+} channel; hESC-CM monolayer) [30]
Nisoldipine	$Ca_v1.2$ channel antagonist	<ul style="list-style-type: none"> • Negative inotropic effect (ventricular muscle) [31] • $IC_{50} = 67\text{--}81\text{nM}$ ($Ca_v1.2$; 	<ul style="list-style-type: none"> • Blocked L-type Ca^{2+} current ($1\text{--}2\mu\text{M}$; 13–24 week CMs) [37, 38] 	<ul style="list-style-type: none"> • Inhibition of AP propagation and contraction ($1\mu\text{M}$; hESC-CM EBs) [39]

Drug Name	Target	Effect in Human Adult CMs/Myocardium	Effect in Human Fetal CMs/Myocardium	Effect in hPSC-CMs
		<ul style="list-style-type: none"> transfected cell line) [36] • $IC_{50} = 200\text{--}400\text{nM}$ (inotropic; ventricular muscle) [31] 		
Thapsigargin	SERCA antagonist	<ul style="list-style-type: none"> • Negative inotropic effect [40] • $\uparrow TTP$ and RT_{90} ($1\mu\text{M}$; LV CMs) [41] • Negative inotropic effect at high frequency, resulting in flattened FFR ($1\mu\text{M}$; LV CMs) [41] 		<ul style="list-style-type: none"> • $\downarrow Ca^{2+}$ transient amplitude ($1\text{--}20\mu\text{M}$; hiPSC-CMs) [42] • $\downarrow Ca^{2+}$ transient amplitude in subset of cells ($0.5\mu\text{M}$; 35–40%; hESC-CMs) [43] • $\downarrow$maximal Ca^{2+} decay velocity ($0.5\mu\text{M}$; hESC-CMs) [43]
Caffeine	Ryanodine receptor agonist	<ul style="list-style-type: none"> • Positive inotropic and chronotropic effect [44] • Left-shifted force-Ca^{2+} relationship (10mM; RV trabecular skinned fiber and intact muscle) [3] • Positive inotropic effect ($>0.5\text{mM}$) and $\uparrow +dF/dt$ (20mM; RV skinned fibers) [45] 	<ul style="list-style-type: none"> • $\uparrow Ca^{2+}$ transient amplitude in subset of cells (65%; 10mM; 16–18 week LV CMs) [43] 	<ul style="list-style-type: none"> • $\uparrow Ca^{2+}$ transient amplitude ($10\text{--}40\text{mM}$; hiPSC-CMs) [42] • $\uparrow Ca^{2+}$ transient amplitude in subset of cells ($35\text{--}40\%$; 10mM; hESC-CMs) [43]
E-4031	$K_{V11.1}$ (hERG) channel antagonist	<ul style="list-style-type: none"> • $\uparrow APD$ [46, 47] • $\downarrow I_K$ current amplitude ($5\mu\text{M}$; RV CMs) [47] • $IC_{50} = 7\text{--}32\text{nM}$ ($K_{V11.1}$; transfected cell line) [22, 25] 	<ul style="list-style-type: none"> • Gestation-dependent effect on AP propagation ($1\mu\text{M}$; no effect on APD in week 5 CMs; $\uparrow APD$ in week 7.5–9.5 CMs) [48] • Irregular AP pattern ($1\mu\text{M}$; week 5–9.5 CMs) [48] • $\downarrow AP$ amplitude and $\uparrow$inter AP interval ($1\mu\text{M}$; 	<ul style="list-style-type: none"> • $\uparrow APD$, EADs and $\uparrow$short-term variability in APD ($1\mu\text{M}$; hESC-CMs) [49] • $\uparrow APD$ and EADs (12nM; hiPSC-CM clusters) [50]

Drug Name	Target	Effect in Human Adult CMs/Myocardium	Effect in Human Fetal CMs/Myocardium	Effect in hPSC-CMs
			week 5–7 fetal CMs) [48]	
Terfenadine	K ⁺ , Na ⁺ , and Ca ²⁺ cardiac ion channel antagonist	<ul style="list-style-type: none"> • $\downarrow I_K$ current, $\uparrow$ rate of current decay and $\downarrow$ current amplitude (3 μM; K_V1.5; transfected cell line, whole cell) [51] • IC₅₀ = 367 nM (K_V1.5; transfected cell line, cell patch) [51] • IC₅₀ = 56 nM (K_V11.1; transfected cell line) [52] • IC₅₀ = 26 nM (K_V11.1; atrial CMs) [53] • Blocked I_{Kr} and I_{to} currents (atrial CMs and K_V1.5 transfected cell line) [54] • Frequency-dependent inhibition of L-type calcium current (IC₅₀ = 185 nM at 1 Hz; IC₅₀ = 750 nM at 0.2 Hz; RA CMs) [55] • $\uparrow$ Na⁺/Ca²⁺-exchange current (I_{NCX}) frequency (1 μM; atrial CMs) [55] • IC₅₀ = 1.7–8.1 μM (Na_V1.5; atrial CMs) [53] 		<ul style="list-style-type: none"> • $\uparrow$ APD (30 nM; hiPSC-CMs) [35] • $\uparrow$ FPD (100 nM; hiPSC-CM monolayer) [22] • $\downarrow$ AP amplitude with (1 μM; hiPSC-CM monolayer) [22] • Negative chronotropic effect (0.01–0.3 μM; hiPSC-CM monolayer) [28] • Time- and dose-dependent irregular AP pattern (0.3 μM; hiPSC-CM monolayer) [28] • Blocked AP propagation (1 μM; hiPSC-CM monolayer) [28]
Tetrodotoxin	Na _V 1.5 channel antagonist	<ul style="list-style-type: none"> • Negative chronotropic effect [56, 57] • $\uparrow$ APD, $\downarrow$ V_{max} (2 μM; papillary muscle) [58] 	<ul style="list-style-type: none"> • Insensitive to TTX (10 μM; 10 week ventricular CMs) [61] • Cessation of contraction (0.63 μM; 13– 	<ul style="list-style-type: none"> • Blocked AP propagation in a subset of cells (100 μM; hiPSC-CMs) [34] • Blocked AP propagation in most cells

Drug Name	Target	Effect in Human Adult CMs/Myocardium	Effect in Human Fetal CMs/Myocardium	Effect in hPSC-CMs
		<ul style="list-style-type: none"> • IC_{50} 1μM [59, 60] 	15 week left atria) [62]	(10 μ M; hESC-CMs) [34]
Lidocaine	Na ⁺ channel antagonist	<ul style="list-style-type: none"> • $\uparrow$APD, $\downarrow$Vmax (ventricular muscle) [63] • IC_{50} = 34–38μM (Na⁺ current; transfected cell line) [64] 		<ul style="list-style-type: none"> • Blocked Na⁺ influx, $\downarrow$conduction velocity, no effect on APD (hiPSC-CMs) [29] • Blocked AP propagation (50μM hiPSC-CMs) [34] • Blocked AP propagation (1mM; hESC-CMs) [34] • $\downarrow$Vmax, $\uparrow$APD and caused MDP depolarization (500μM; hESC-CMs) [34]

LV = left ventricle; RV = right ventricle; RA = right atria; AP = action potential; APD = action potential duration; FPD = field potential duration; FFR = force-frequency relationship; $+dF/dt$ = maximal rate of force increase; EAD = early afterdepolarization; Vmax = depolarization velocity; MDP = maximum diastolic potential

- [1] S. Weiss, S. Oz, A. Benmocha, N. Dascal, Regulation of cardiac L-type Ca(2+)(+) channel CaV1.2 via the beta-adrenergic-cAMP-protein kinase A pathway: old dogmas, advances, and new uncertainties, *Circ. Res.* 113 (2013) 617–631.
- [2] E.G. Kranias, R.J. Solaro, Phosphorylation of troponin I and phospholamban during catecholamine stimulation of rabbit heart, *Nature* 298 (1982) 182–184.
- [3] J.K. Gwathmey, R.J. Hajjar, Relation between steady-state force and intracellular [Ca2+] in intact human myocardium. Index of myofibrillar responsiveness to Ca2+, *Circulation* 82 (1990) 1266–1278.
- [4] M. Flesch, R.H. Schwinger, F. Schiffer, K. Frank, M. Sudkamp, F. Kuhn-Regnier, G. Arnold, M. Bohm, Evidence for functional relevance of an enhanced expression of the Na(+)-Ca2+ exchanger in failing human myocardium, *Circulation* 94 (1996) 992–1002.
- [5] C. Holubarsch, R. Schneider, B. Pieske, T. Ruf, G. Hasenfuss, G. Fraedrich, H. Posival, H. Just, Positive and negative inotropic effects of DL-sotalol and D-sotalol in failing and nonfailing human myocardium under physiological experimental conditions, *Circulation* 92 (1995) 2904–2910.
- [6] M. Bohm, I. Morano, B. Pieske, J.C. Ruegg, M. Wankel, R. Zimmermann, E. Erdmann, Contribution of cAMP-phosphodiesterase inhibition and sensitization of the contractile proteins for calcium to the inotropic effect of pimobendan in the failing human myocardium, *Circ. Res.* 68 (1991) 689–701.
- [7] M. White, R. Roden, W. Minobe, M.F. Khan, P. Larrabee, M. Wollmering, J.D. Port, F. Anderson, D. Campbell, A.M. Feldman, Age-related changes in beta-adrenergic neuroeffector systems in the human heart, *Circulation* 90 (1994) 1225–1238.
- [8] M. Bohm, K. La Rosee, R.H. Schwinger, E. Erdmann, Evidence for reduction of norepinephrine uptake sites in the failing human heart, *J. Am. Coll. Cardiol.* 25 (1995) 146–153.
- [9] D.J. Coltart, B.A. Spilker, Development of human foetal inotropic responses to catecholamines, *Experientia* 28 (1972) 525–526.
- [10] M. Toraason, D.E. Richards, P.I. Mathias, Ca2+ mobilization in fetal-human cardiac myocytes is stimulated by isoproterenol and inhibited by ryanodine, *In Vitro Cell. Dev. Biol. Anim.* 34 (1998) 19–21.

- [11] B.A. Resch, J.G. Papp, Effect of adrenaline, noradrenaline, isoproterenol and tyramine on the isolated surviving human fetal heart, *Zentralbl. Gynakol.* 104 (1982) 1451–1461.
- [12] F. Pillekamp, M. Hausteine, M. Khalil, M. Emmelheinz, R. Nazzari, R. Adelmann, F. Nguemo, O. Rubenchyk, K. Pfannkuche, M. Matzkies, M. Reppel, W. Bloch, K. Brockmeier, J. Hescheler, Contractile properties of early human embryonic stem cell-derived cardiomyocytes: beta-adrenergic stimulation induces positive chronotropy and lusitropy but not inotropy, *Stem Cells Dev.* 21 (2012) 2111–2121.
- [13] I.C. Turnbull, I. Karakikes, G.W. Serrao, P. Backeris, J.J. Lee, C. Xie, G. Senyei, R.E. Gordon, R.A. Li, F.G. Akar, R.J. Hajjar, J.S. Hulot, K.D. Costa, Advancing functional engineered cardiac tissues toward a preclinical model of human myocardium, *FASEB J.* 28 (2014) 644–654.
- [14] C. Mummery, D. Ward-van Oostwaard, P. Doevendans, R. Spijker, S. van den Brink, R. Hassink, M. van der Heyden, T. Ophof, M. Pera, A.B. de la Riviere, R. Passier, L. Tertoolen, Differentiation of human embryonic stem cells to cardiomyocytes: role of coculture with visceral endoderm-like cells, *Circulation* 107 (2003) 2733–2740.
- [15] M. Brito-Martins, S.E. Harding, N.N. Ali, Beta(1)- and Beta(2)-Adrenoceptor Responses in Cardiomyocytes Derived from Human Embryonic Stem Cells: Comparison with Failing and Non-Failing Adult Human Heart, *Br. J. Pharmacol.* 153 (2008) 751–759.
- [16] R.L. Bieganski, A.J. Pappano, Dual mechanism for inhibition of calcium-dependent action potentials by acetylcholine in avian ventricular muscle. Relationship to cyclic AMP, *Circ. Res.* 46 (1980) 353–362.
- [17] A.J. Pappano, P.M. Hartigan, M.D. Coutu, Acetylcholine inhibits positive inotropic effect of cholera toxin in ventricular muscle, *Am. J. Physiol.* 243 (1982) H434–441.
- [18] W. Von Scheidt, M. Bohm, A. Stablein, G. Autenrieth, E. Erdmann, Antiadrenergic effect of M-cholinoceptor stimulation on human ventricular contractility in vivo, *Am. J. Physiol.* 263 (1992) H1927–31.
- [19] J. Koglin, M. Bohm, W. von Scheidt, A. Stablein, E. Erdmann, Antiadrenergic effect of carbachol but not of adenosine on contractility in the intact human ventricle in vivo, *J. Am. Coll. Cardiol.* 23 (1994) 678–683.
- [20] D.J. Coltart, B.A. Spilker, S.J. Meldrum, An electrophysiological study of human foetal cardiac muscle, *Experientia* 27 (1971) 797–799.
- [21] S. Zhang, Z. Zhou, Q. Gong, J.C. Makielski, C.T. January, Mechanism of block and identification of the verapamil binding domain to HERG potassium channels, *Circ. Res.* 84 (1999) 989–998.
- [22] K. Harris, M. Aylott, Y. Cui, J.B. Louttit, N.C. McMahon, A. Sridhar, Comparison of electrophysiological data from human-induced pluripotent stem cell-derived cardiomyocytes to functional preclinical safety assays, *Toxicol. Sci.* 134 (2013) 412–426.
- [23] B.S. Freeze, M.M. McNulty, D.A. Hanck, State-dependent verapamil block of the cloned human Ca(v)3.1 T-type Ca(2+) channel, *Mol. Pharmacol.* 70 (2006) 718–726.
- [24] Y.A. Kuryshov, A.M. Brown, E. Duzic, G.E. Kirsch, Evaluating state dependence and subtype selectivity of calcium channel modulators in automated electrophysiology assays, *Assay Drug Dev. Technol.* 12 (2014) 110–119.
- [25] K. Yamazaki, T. Hihara, H. Kato, T. Fukushima, K. Fukushima, T. Taniguchi, T. Yoshinaga, N. Miyamoto, M. Ito, K. Sawada, Beat-to-Beat Variability in Field Potential Duration in Human Embryonic Stem Cell-Derived Cardiomyocyte Clusters for Assessment of Arrhythmogenic Risk, and a Case Study of Its Application, *Pharmacology and Pharmacy* 5 (2014) 117–128.
- [26] R.H. Schwinger, M. Bohm, E. Erdmann, Different negative inotropic activity of Ca2(+)-antagonists in human myocardial tissue, *Klin. Wochenschr.* 68 (1990) 797–805.
- [27] D. Sarsero, T. Fujiwara, P. Molenaar, J.A. Angus, Human vascular to cardiac tissue selectivity of L- and T-type calcium channel antagonists, *Br. J. Pharmacol.* 125 (1998) 109–119.
- [28] L. Guo, R.M. Abrams, J.E. Babiarz, J.D. Cohen, S. Kameoka, M.J. Sanders, E. Chiao, K.L. Kolaja, Estimating the risk of drug-induced proarrhythmia using human induced pluripotent stem cell-derived cardiomyocytes, *Toxicol. Sci.* 123 (2011) 281–289.
- [29] A. Mehta, Y. Chung, G.L. Sequiera, P. Wong, R. Liew, W. Shim, Pharmacoelectrophysiology of viral-free induced pluripotent stem cell-derived human cardiomyocytes, *Toxicol. Sci.* 131 (2013) 458–469.
- [30] J. Kang, X.L. Chen, J. Ji, Q. Lei, D. Rampe, Ca2(+) channel activators reveal differential L-type Ca2(+) channel pharmacology between native and stem cell-derived cardiomyocytes, *J. Pharmacol. Exp. Ther.* 341 (2012) 510–517.
- [31] T. Godfraind, C. Egleme, M. Finet, P. Jaumin, The actions of nifedipine and nisoldipine on the contractile activity of human coronary arteries and human cardiac tissue in vitro, *Pharmacol. Toxicol.* 61 (1987) 79–84.

- [32] T.K. Chin, K.S. Graham, C. Calendine, Y.H. He, Neuroregulation of Calcium Channel Function in Human Fetal Cardiac Myocyte Clusters, *Pediatric Research* 45 (1999) 21A.
- [33] G. Bkaily, N. El-Bizri, M. Bui, R. Sukarieh, D. Jacques, M.L. Fu, Modulation of intracellular Ca^{2+} via L-type calcium channels in heart cells by the autoantibody directed against the second extracellular loop of the α_1 -adrenoceptors, *Can. J. Physiol. Pharmacol.* 81 (2003) 234–246.
- [34] X. Sheng, M. Reppel, F. Nguemo, F.I. Mohammad, A. Kuzmenkin, J. Hescheler, K. Pfannkuche, Human pluripotent stem cell-derived cardiomyocytes: response to TTX and lidocaine reveals strong cell to cell variability, *PLoS One* 7 (2012) e45963.
- [35] J.K. Gibson, Y. Yue, J. Bronson, C. Palmer, R. Numann, Human stem cell-derived cardiomyocytes detect drug-mediated changes in action potentials and ion currents, *J. Pharmacol. Toxicol. Methods* 70 (2014) 255–267.
- [36] I. Splawski, K.W. Timothy, L.M. Sharpe, N. Decher, P. Kumar, R. Bloise, C. Napolitano, P.J. Schwartz, R.M. Joseph, K. Condouris, H. Tager-Flusberg, S.G. Priori, M.C. Sanguinetti, M.T. Keating, $\text{Ca(V)}_{1.2}$ calcium channel dysfunction causes a multisystem disorder including arrhythmia and autism, *Cell* 119 (2004) 19–31.
- [37] L. Chen, N. El-Sherif, M. Boutjdir, Unitary current analysis of L-type Ca^{2+} channels in human fetal ventricular myocytes, *J. Cardiovasc. Electrophysiol.* 10 (1999) 692–700.
- [38] M. Boutjdir, L. Chen, Z.H. Zhang, C.E. Tseng, F. DiDonato, W. Rashbaum, A. Morris, N. el-Sherif, J.P. Buyon, Arrhythmogenicity of IgG and anti-52-kD SSA/Ro affinity-purified antibodies from mothers of children with congenital heart block, *Circ. Res.* 80 (1997) 354–362.
- [39] S. Schaaf, A. Shibamiya, M. Mewe, A. Eder, A. Stohr, M.N. Hirt, T. Rau, W.H. Zimmermann, L. Conradi, T. Eschenhagen, A. Hansen, Human engineered heart tissue as a versatile tool in basic research and preclinical toxicology, *PLoS One* 6 (2011) e26397.
- [40] B.J. Poindexter, J.R. Smith, L.M. Buja, R.J. Bick, Calcium signaling mechanisms in dedifferentiated cardiac myocytes: comparison with neonatal and adult cardiomyocytes, *Cell Calcium* 30 (2001) 373–382.
- [41] K. Davia, C.H. Davies, S.E. Harding, Effects of inhibition of sarcoplasmic reticulum calcium uptake on contraction in myocytes isolated from failing human ventricle, *Cardiovasc. Res.* 33 (1997) 88–97.
- [42] I. Itzhaki, S. Rapoport, I. Huber, I. Mizrahi, L. Zwi-Dantsis, G. Arbel, J. Schiller, L. Gepstein, Calcium handling in human induced pluripotent stem cell derived cardiomyocytes, *PLoS One* 6 (2011) e18037.
- [43] J. Liu, J.D. Fu, C.W. Siu, R.A. Li, Functional sarcoplasmic reticulum for calcium handling of human embryonic stem cell-derived cardiomyocytes: insights for driven maturation, *Stem Cells* 25 (2007) 3038–3044.
- [44] M.G. Khana, Chapter 31: Caffeine and the Heart, in: M.G. Khana (Ed.), **Encyclopedia of Heart Diseases**, Elsevier Academic Press, Burlington, MA, 2006, pp. 189–191.
- [45] A. D'Agnolo, G.B. Luciani, A. Mazzucco, V. Gallucci, G. Salvati, Contractile properties and Ca^{2+} release activity of the sarcoplasmic reticulum in dilated cardiomyopathy, *Circulation* 85 (1992) 518–525.
- [46] L. Guo, J.Y. Qian, R. Abrams, H.M. Tang, T. Weiser, M.J. Sanders, K.L. Kolaja, The electrophysiological effects of cardiac glycosides in human iPSC-derived cardiomyocytes and in guinea pig isolated hearts, *Cell. Physiol. Biochem.* 27 (2011) 453–462.
- [47] G.R. Li, J. Feng, L. Yue, M. Carrier, S. Nattel, Evidence for two components of delayed rectifier K^{+} current in human ventricular myocytes, *Circ. Res.* 78 (1996) 689–696.
- [48] C. Danielsson, J. Brask, A.C. Skold, R. Genead, A. Andersson, U. Andersson, K. Stockling, R. Pehrson, K.H. Grinnemo, S. Salari, H. Hellmold, B. Danielsson, C. Sylven, F. Elinder, Exploration of human, rat, and rabbit embryonic cardiomyocytes suggests K^{+} -channel block as a common teratogenic mechanism, *Cardiovasc. Res.* 97 (2013) 23–32.
- [49] L. Nalos, R. Varkevisser, M.K. Jonsson, M.J. Houtman, J.D. Beekman, R. van der Nagel, M.B. Thomsen, G. Duker, P. Sartipy, T.P. de Boer, M. Peschar, M.B. Rook, T.A. van Veen, M.A. van der Heyden, M.A. Vos, Comparison of the IKr blockers moxifloxacin, dofetilide and E-4031 in five screening models of pro-arrhythmia reveals lack of specificity of isolated cardiomyocytes, *Br. J. Pharmacol.* 165 (2012) 467–478.
- [50] D.K. Jones, F. Liu, R. Vaidyanathan, L.L. Eckhardt, M.C. Trudeau, G.A. Robertson, hERG 1b is critical for human cardiac repolarization, *Proc. Natl. Acad. Sci. U. S. A.* 111 (2014) 18073–18077.
- [51] D. Rampe, B. Wible, A.M. Brown, R.C. Dage, Effects of terfenadine and its metabolites on a delayed rectifier K^{+} channel cloned from human heart, *Mol. Pharmacol.* 44 (1993) 1240–1245.
- [52] A.E. Lacerda, J. Kramer, K.Z. Shen, D. Thomas, A.M. Brown, Comparison of block among cloned cardiac potassium channels by non-antiarrhythmic drugs, *European Heart Journal* 3 (2001) K23–K30.

- [53] H.R. Lu, A.N. Hermans, D.J. Gallacher, Does terfenadine-induced ventricular tachycardia/fibrillation directly relate to its QT prolongation and Torsades de Pointes? *Br. J. Pharmacol.* 166 (2012) 1490–1502.
- [54] W.J. Crumb Jr, B. Wible, D.J. Arnold, J.P. Payne, A.M. Brown, Blockade of multiple human cardiac potassium currents by the antihistamine terfenadine: possible mechanism for terfenadine-associated cardiotoxicity, *Mol. Pharmacol.* 47 (1995) 181–190.
- [55] L. Hove-Madsen, A. Llach, C.E. Molina, C. Prat-Vidal, J. Farre, S. Roura, J. Cinca, The proarrhythmic antihistaminic drug terfenadine increases spontaneous calcium release in human atrial myocytes, *Eur. J. Pharmacol.* 553 (2006) 215–221.
- [56] M. Baruscotti, A. Bucchi, D. DiFrancesco, Physiology and pharmacology of the cardiac pacemaker (“funny”) current, *Pharmacol. Ther.* 107 (2005) 59–79.
- [57] Y.K. Ju, P.W. Gage, D.A. Saint, Tetrodotoxin-sensitive inactivation-resistant sodium channels in pacemaker cells influence heart rate, *Pflugers Arch.* 431 (1996) 868–875.
- [58] L. Barandi, L. Virag, N. Jost, Z. Horvath, I. Koncz, R. Papp, G. Harmati, B. Horvath, N. Szentandrassy, T. Banyasz, J. Magyar, A. Zaza, A. Varro, P.P. Nanasi, Reverse rate-dependent changes are determined by baseline action potential duration in mammalian and human ventricular preparations, *Basic Res. Cardiol.* 105 (2010) 315–323.
- [59] W.A. Catterall, A.L. Goldin, S.G. Waxman, International Union of Pharmacology. XLVII. Nomenclature and structure-function relationships of voltage-gated sodium channels, *Pharmacol. Rev.* 57 (2005) 397–409.
- [60] A.L. Goldin, Resurgence of sodium channel research, *Annu. Rev. Physiol.* 63 (2001) 871–894.
- [61] D. Jacques, G. Bkaily, G. Jasmin, D. Menard, L. Proschek, Early fetal like slow Na⁺ current in heart cells of cardiomyopathic hamster, *Mol. Cell. Biochem.* 176 (1997) 249–256.
- [62] D. Walker, Functional development of the autonomic innervation of the human fetal heart, *Biol. Neonate* 25 (1974) 31–43.
- [63] K.A. Kane, Comparative electrophysiological effects of Org 6001, a new orally active antidysrhythmic agent, and lignocaine on human ventricular muscle, *Br. J. Pharmacol.* 68 (1980) 25–31.
- [64] P.B. Bennett, C. Valenzuela, L.Q. Chen, R.G. Kallen, On the molecular nature of the lidocaine receptor of cardiac Na⁺ channels. Modification of block by alterations in the alpha-subunit III–IV interdomain, *Circ. Res.* 77 (1995) 584–592.