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Review article

# Lessons from the heart: Mirroring electrophysiological characteristics during cardiac development to in vitro differentiation of stem cell derived cardiomyocytes

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ABSTRACT

The ability of human pluripotent stem cells (hPSCs) to differentiate into any cell type of the three germ layers makes them a very promising cell source for multiple purposes, including regenerative medicine, drug discovery, and as a model to study disease mechanisms and progression. One of the first specialized cell types to be generated from hPSC was cardiomyocytes (CM), and differentiation protocols have evolved over the years and now allow for robust and large-scale production of hPSC-CM. Still, scientists are struggling to achieve the same, mainly ventricular, phenotype of the hPSC-CM in vitro as their adult counterpart in vivo. In vitro generated cardiomyocytes are generally described as fetal-like rather than adult. In this review, we compare the in vivo development of cardiomyocytes to the in vitro differentiation of hPSC into CM with focus on electrophysiology, structure and contractility. Furthermore, known epigenetic changes underlying the differences between adult human CM and CM differentiated from pluripotent stem cells are described. This should provide the reader with an extensive overview of the current status of human stem cell-derived cardiomyocyte phenotype and function. Additionally, the reader will gain insight into the underlying signaling pathways and mechanisms responsible for cardiomyocyte development.



**Contents**



*Abbreviations:* AP, action potential; CICR, calcium induced calcium release; CM, cardiomyocyte; hESC, human embryonic stem cell; hiPSC, human induced pluripotent stem cell; hPSC, human pluripotent stem cell (either hESC and/or hiPSC); HF, heart failure; $I_{Ca,L}$, L-type calcium current; $I_{Ca,T}$, T-type calcium current; $I_f$, funny or pacemaker current; $I_{K1}$, inward rectifier potassium current; $I_{K,Ach}$, acethylcholine activated potassium current; $I_{K,ATP}$, ATP sensitive potassium current; $I_{Kr}$, rapid delayed rectifier potassium current; $I_{Kur}$, ultrarapid delayed rectifier potassium current; $I_{Ks}$, slow delayed rectifier potassium current; $I_{Na}$, sodium current; $I_{to}$, transient outward potassium current; lncRNA, long non-coding RNA; MI, myocardial infarction; miRNA, microRNA; RMP, resting membrane potential; SR, sarcoplasmatic reticulum.

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## 1. Introduction

The ability of human embryonic stem cells (hESCs) to differentiate into any cell type of the three germ layers makes them a very promising cell source for multiple purposes [1]. The use of stem cells within the cardiovascular field has long been focused on their regenerative properties and their potential to treat cardiovascular diseases such as myocardial infarction (MI) and heart failure (HF). However, preclinical animal studies and clinical trials have shown mixed results [2]. Even though some early non-controlled pilot studies found improved cardiac function upon stem cell transplantation, recent randomized and controlled trials failed to find similar results. Patients enrolled in these randomized controlled trials showed either an unaltered ejection fraction (EF) or displayed an increase in EF which was too low to exceed the minimal change needed for long-term improvement of symptoms and survival [3–8]. Potential reasons for these mixed results can be the age of the patients, timing between treatments, occurrence of the MI, method of cell transplantation and the cell type used [9–11]. The most often used cell types have been adult stem cells, such as mesenchymal cells and endothelial progenitor cells derived from bone-marrow [12].

Next to their applicability in regenerative medicine, human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs), including both embryonic (hESC-CMs) and induced pluripotent (hiPSC-CMs) stem cell-derived cardiomyocytes, are a promising future in vitro cellular model to study cardiac arrhythmia-related diseases and for screening of pro-arrhythmic and cardiotoxic compounds during drug development [13,14]. Introducing human stem cells into routine drug development could reduce the necessity for animal testing, which is both ethically sensitive and costly. Moreover, it would circumvent problems with interspecies differences that impede the translation of the results to human. To be useful as a screening model, hPSC-CM should resemble human adult CM as closely as possible with regard to structure, excitation (through action potential (AP) generation) and contraction (largely dependent upon $Ca^{2+}$ cycling). CMs resembling those of the ventricles are typically wanted for safety pharmacology testing since this is where the most severe, and potentially lethal, arrhythmias occur. In this review we will primarily focus on the electrophysiological development and function of cardiomyocytes in vivo and in vitro, but beyond that also touch upon structure and contractility.

### 1.1. Background

Though several aspects of hPSC-CMs are comparable to those of adult CMs, important differences exist. hPSC-CMs are spontaneously beating cells with unorganized sarcomeres and have a different ion channel profile when compared to adult CMs. hPSC-CMs typically have APs with a relatively depolarized resting membrane potential (RMP) compared to adult CM, independently of which line and differentiation protocol is being used. The underlying cause of this difference is most likely a lack of the inward rectifier potassium current ($I_{K1}$), whose role is to hyperpolarize and stabilize the RMP of the cell [14,15] (Fig. 1). This notion is supported by several recent studies using a variety of strategies including modeling, over-expression of the pore-forming protein and electronic addition of $I_{K1}$ through dynamic clamp, all leading to drastically improved AP shape and characteristics [16–18]. Previous work showed that ion channel expression and ion currents in hESC-CM undergo developmental maturation over time, measured as modifications in current densities and properties [15]. This has been supported by more recent studies in which cells cultured for an extended time period show increased expression of ion channels and significantly improved cell morphology [19–21]. In none of these studies, however, did AP shape or $I_{K1}$ expression come close to that of adult CM. Moreover, although hPSC-CMs are capable of contracting, both spontaneously and in response to electrical stimulation, their excitation–contraction machinery is immature. In particular, hPSC-CMs lack clear T-tubuli, have disorganized sarcomeric striations and immature $Ca^{2+}$ handling [22–27]. Over-expressing one of the genes responsible for part of the $Ca^{2+}$-handling machinery (CASQ2) led to more mature calcium transients [28]. All together, the phenotype and functionality of hPSC-CM more closely resembles that of a fetal than an adult CM, and this currently hampers their optimal applicability in regenerative medicine, drug screening and as a model to study cardiac arrhythmia-related diseases.

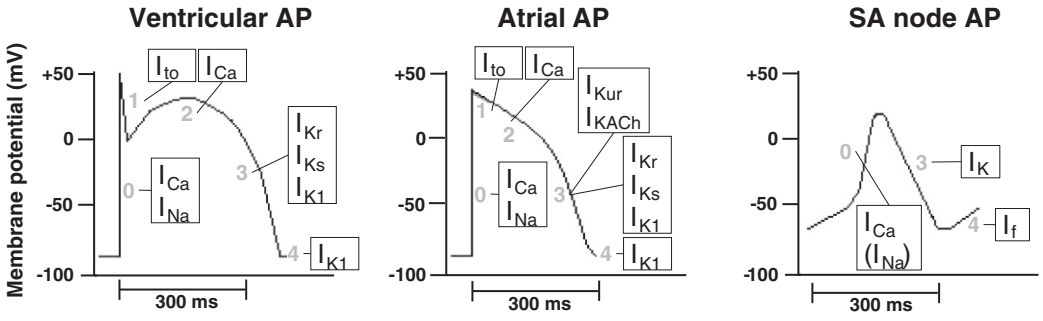


**Fig. 1.** Schematic showing the cardiac action potential (AP) from different regions of the heart. To the left, a typical AP from a ventricular CM with a stable resting membrane potential, rapid upstroke and pronounced plateau phase. In the middle, an AP from an atrial CM which is similar to the one in the ventricle but with a less pronounced plateau, faster repolarization and consequently shorter AP duration. To the right, an AP from a SA nodal cell with typical phase 4 depolarization, slow upstroke and lack of clear plateau phase. The different phases of each AP are indicated with gray numbers and the major underlying ion currents are noted.