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Circulation. Author manuscript; available in PMC 2012 March 6.

Published in final edited form as:

Circulation. 2009 December 15; 120(24): 2496–2508. doi:10.1161/CIRCULATIONAHA.107.751412.

Electrophysiological challenges of cell-based myocardial repair

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Keywords

automaticity; trigger; reentry

Cardiovascular diseases remain the major cause of death in the western world.[1] Stem and progenitor cell (SPC)-based therapies in animal models and human trials in recent years have suggested promising therapeutic potential and drawn intense public interest. Possible beneficial mechanisms of cell-based therapies include generation of new and mature ventricular cardiomyocytes (cardiomyogenesis), recruitment of endogenous SPCs for cardiomyogenesis, and salvage of native myocardium through paracrine, angiogenic, and anti-apoptotic effects. [2, 3] We refer readers to several excellent reviews for an overview of cell-based therapies for cardiac diseases.[2–5] Here we discuss potential electrophysiological challenges and arrhythmic risks posed by cell therapies designed to replace damaged myocardium. Although adverse outcomes after cell therapies, such as lethal arrhythmias, occurred in early clinical trials of skeletal myoblast (SkM) transplantation, [5–8] recent clinical trials using various sources of SPCs, novel modes of cell delivery, better cell selection, and different timing of cell transplantation did not show any significant increase in arrhythmic incidence. It is tempting to conclude on this basis that cell-based therapies are "safe" and non-arrhythmogenic (reviewed in [2, 4]); however, the negligible production of new cardiomyocytes (CMs) documented for many SPCs employed make it difficult to extrapolate their safety records towards future trials in which substantial numbers of new CMs might be generated or introduced. Clinical trials to test cardiomyocyte replacement (cardiomyogenesis), long a goal of SPC-based therapy, are bound to take place in the near future, thanks to many investigators who are developing technologies for efficient generation of CMs from SPCs and for increasing their retention after transplantation. In weighing the benefits of replacement therapies, we should not overlook the fact that SPC-derived CMs (SPC-CMs) exhibit variable electrophysiological (EP) properties and are typically immature as compared to adult CMs (see below). This immaturity of primitive SPC-CMs remains a major hurdle towards developing safe cell therapies. In anticipation of improved cardiomyogenesis in future cell therapies, we will review here developmental and electrophysiological challenges, common to all SPC-CMs, that need to be resolved in order to improve the maturation of primitive SPC-CMs and to avoid unwanted risks of increasing arrhythmic incidence (pro-arrhythmia) after transplantation.

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Section Editor: Dr. Calum A. Macrae

Disclosures: Dr. Chen and Dr. Mercola received research grants from agencies listed in funding sources. Dr. Kim has nothing to disclose.

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Concerns about Maturation Status of Transplanted Cells

Lack of Regional and Timely Developmental Cues for Subtype Specification and Maturation

Research in cardiac morphogenesis has provided valuable insights into basic mechanisms controlling cardiomyogenesis, and these are beginning to be applied to direct differentiation of cardiomyocytes from SPCs (reviewed in [9–13]). Cardiomyocytes develop from cardiogenic mesoderm (Nkx2.5+ and Mesp1/2+) during embryonic development. In addition to the primary cardiac mesoderm, there are other sources of cells that are recruited to join the mature myocardium during cardiac development, including a second wave of cardiogenic mesoderm, known as the secondary heart field, that gives rise to the outflow tract, right ventricle, and portions of the atria.[11, 14] Cardiomyocytes in the developing fetal heart continue to proliferate, despite functional contraction, but mitotic activity declines quickly after birth (few weeks in rat) [15] and continued growth of the heart is largely due to an increase in myocyte size.[9] In human, cardiomyocytes reach adult size at ages between 10 to 20 years.[16] The prevailing view that the heart does not regenerate has slowly given way to the idea that some degree of endogenous regeneration occurs through replacement of dead and damaged cells, in particular after injury.[9, 17] Three aspects of cardiac development are relevant to cell replacement therapy: differentiation, subtype-specification, and maturation of cardiomyocytes. In a simple schematic, Figure 1 illustrates some of many factors and genes that work in a coordinated, region-specific and temporally defined fashion to determine the fate of various subtypes of cardiomyocytes during cardiogenesis. Not detailed are various extra-cardiac cells, such as pharyngeal mesodermal cells, mesenchymal cells at arterial and venous poles, and possibly circulating hematopoietic and/or endothelial progenitor cells (HPCs and/or EPCs), all contributing to or influencing the formation of a functional myocardium. During early development when many of the cell lineage decisions are being made, a number of regulatory factors are diffusible proteins derived from other tissues, such as pharyngeal endoderm, neural crest and pro-epicardium. A number of key factors that dictate chamber identity are diagrammed in Figure 2, illustrating that many subtle temporal and regional cues determine the subtype specification of cardiomyocytes, such as atrial, ventricular and pacemaking CMs, and direct their maturation during normal cardiac development.[18–19] How these regional and temporal cues influence the expression of various types of ion channels remains to be explored.

Most cellular cardiomyocyte replacement strategies have focused on using immature cardiomyocytes or committed progenitors since the adult heart seems unlikely to produce the complex array of signaling molecules needed to direct cardiogenic differentiation of uncommitted SPCs. Embryonic stem cells are the extreme case in that they are derived from and resemble cells of pre-implantation embryos, form teratomas upon transplantation, and appear to require a recapitulation of the cardiogenic differentiation program that occurs in the developing embryo to yield CMs. [3, 20] It is unrealistic to expect the adult heart to provide such a vast array of developmental cues, and even less so in the correct sequence. Whether the damaged adult heart produces factors [21] capable of stimulating clinically meaningful differentiation of extra-cardiac SPCs, such as HPCs, also remains controversial. [3] Studies reporting induced differentiation of extra-cardiac SPCs by juxtaposition with adult myocardium *in vitro* or after implantation *in vivo* have largely relied on co-localizing cardiomyocyte immunostaining with lineage labels, yet discrepancies in experimental protocols and estimates of differentiation efficiency vary to the extent that the premise of transdifferentiation of extra-cardiac SPCs into CMs is a matter of considerable dispute.[3] Therefore, considering the broader picture, it is clear that the question of the optimal developmental stage to implant cells remains unresolved for most SPCs and that the answer is likely to vary depending on the particular SPC. Transplanting uncommitted cells could result in poor differentiation, or even form a tumor, as in the case of highly proliferative

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cells such as embryonic stem cells (ESCs). Since late stage differentiation to a fully matured VM population has not been achieved (see below), and these cells are probably too fragile to survive implantation, it would seem, therefore, that immature cardiomyocytes or committed progenitors offer the most promise. However, the ability of adult myocardium to support further development, maturation and EP subtype specification of such cells remains poorly probed and in all likelihood will need to be studied further to elucidate potential means of post-transplant augmentation in order to avoid unwanted risks.[2–5]

Future Electrophysiological Challenges in Cardiomyogenesis

Gap Junction Distribution Mismatch

Gap junctions, formed by various types of connexins (Cxs) are important for cardiomyocyte coupling and electrical conduction. In adult ventricular cardiomyocytes (VMs), Cxs are mostly confined to intercalated discs for longitudinal conduction.[22] In contrast, Cxs in normal neonatal VMs and, pathologically, at border zones of myocardial infarction (MI) distribute over the entire perimeter of myocytes in a punctate pattern.[23,24] It takes 6 years for normal human neonatal myocytes to completely acquire the adult pattern of gap junction distribution.[23] Additionally, pathologically disturbed distribution of gap junctions has been linked to arrhythmogenesis in MI, ventricular hypertrophy, atrial fibrillation and aging hearts.[25] Not surprisingly, gap junction distribution of SPC-CMs resembles that of neonatal cardiomyocytes (Figure 3), which have slower conduction velocities.[26] Moreover, cadherin and gap junction connections between SPC-CMs and host myocytes in most co-cultures and animal models are randomly distributed at the contact interfaces without proper alignment [27–29] (Figure 3). Action potential (AP) propagation across these interfaces is unpredictable, inhomogeneous, and, consequently, might result in increased anisotropy and reentrant arrhythmias. [27] Little is known about the developmental and transcriptional control of myocardial Cx expression, [30] particularly the major Cx of VMs, Cx43. Furthermore, SkM-derived myotubes do not express cadherin or Cx43,[31] and do not electromechanically couple to host myocytes.[32] As a result, conduction slowing and induction of arrhythmia after SkM transplantation have been demonstrated (see below).

More importantly, ion channels, electrical dispersion, and tissue architecture including fibroblasts all contribute to normal and abnormal cardiac conduction.[33] Therefore, it is necessary to examine many other cellular and tissue attributes in order to avoid an over-simplified view of cardiac conduction and to provide a true evaluation of functional integration of SPC-CMs after cell transplantation.

Cell Size and Shape Mismatch

Other than gap junctions, cell size has been shown to be a major determinant of impulse propagation and maximal rate of AP depolarization ($V_{max} = dV/dt_{max}$). [34] Adult VMs have a cylindrical and elongated shape, contracting along a single axis with a positive force-frequency relationship.[35] Neonatal cardiomyocytes and pacemaker cells, however, have a fusiform or spindle-like appearance.[34] In comparison, most SPCs and SPC-CMs used for clinical trials or animal models display irregular cell shapes (neonatal-like, Figure 3) with their mean size (< 50–60 μM) [26,36–37] smaller than adult VMs. [15,34] In contrast, SkM-derived myotubes are larger than VMs in size [38,39] and display fusiform shapes with characteristics of both skeletal and cardiac cells. [40] Also, some SPCs, e.g. hematopoietic stem cells, may not have significant potential for regenerating myocytes but survive in the damaged heart and contribute to other cell types.[3] Therefore, in addition to immature Cx connections between donor and host myocardial cells, cell size mismatch *per se* and subsequent changes in interstitial space and/or resistance could cause slower conduction and depolarization source-sink mismatch, both of which facilitate reentry.[34] Size match and

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cellular alignment of engrafted cells is rarely achieved in most reports, [29,41–42] therefore, translational research into methods for stimulating maturation, alignment and electrical coupling of regenerated cardiomyocytes will be required.[42]

Macroscopic myocardial fiber alignment mismatch

Macroscopically, the left ventricular muscle fibers can be separated into three layers. The superficial layer runs a slightly slanting vertical course, the midlayer fibers form a circular pattern, and the endocardial (deep) layer lies mainly vertically. These three fiber layers work in a coordinate fashion with the supporting fibrous matrix to create a twisting motion for generating efficient cardiac ejection. [43] The alignment of the injected SPC-CMs with the host muscle fibers has been shown to be discordant or, in rare occasions, concordant. [20] Currently, there is no established method to align the injected cells into a single muscle layer. Misaligned muscle fibers could create dyssynchronous contractions, leading to inefficient pumping function, inappropriate stretch and local electrical dispersion. [44]

Acton potential mismatch

Ion channels are important for excitation-contraction (EC) coupling, pulse generation, signal transduction, and cell differentiation/proliferation/maturation.[45] Altered properties of sodium (Na^+), potassium (K^+) and calcium (Ca^{2+}) channels are found in failing hearts and at the epicardial border zone of infarcted myocardium, which has been linked to the stabilization of ventricular tachycardia (VT) circuits. [46,47] The AP duration (APD) in human hearts during the first few years of life increases by about 20% despite a 16-fold increase in heart weight and a 2.4-fold increase in myocardial cell size.[15,48] Adult VMs are usually quiescent (although excitable) and characterized by hyperpolarized maximal diastolic potential (MDP), relatively long APD and the absence of spontaneous phase 4 depolarization. [49]

Table 1 is a literature search summary of data from electrophysiological characterizations of SPC-CMs. Compared to EP properties of adult cardiomyocytes (CMs), Table 1 depicts that most SPC-CMs, at best, exhibit APs similar to fetal or neonatal CMs. [37,50] Regardless of cell sources, these heterogeneous APs are commonly categorized as sinus node- (or pacemaker-), atrial-, and ventricular-like CMs based on overall EP morphologies. [53] Implanting such immature SPC-CMs with variable APDs relative to aging adult CMs may not be beneficial. [71] Furthermore, the ability of HPC or EPCs to form electrophysiologically functional CMs by transdifferentiation remains controversial (discussed in [3]). Other types of extra-cardiac SPCs, such as mesenchymal stem cells (MSCs) from bone marrow (BM), [58] and adipose tissue derived stem cells, [63] have been reported to yield heterogeneous populations of CMs with various AP shapes (see Table 1), albeit at very low incidence. Also, SkM-derived myotubes obtained from cultures or after engraftment into myocardium displayed very short APD, fast afterdepolarization, and bursting neuronal-like activities (similar to trains of early-afterdepolarization in Torsades de Pointes).[32] Thus far, no studies have demonstrated that a uniform and mature EP phenotype could be derived from any SPC-CM *in vitro* or *in vivo* (Table 1). Thus, we expect that the EP heterogeneity of SPC-CMs will likely pose substantial challenges for developing a safe cell replacement therapy. A multidisciplinary evaluation of functional properties of SPC-CMs after cell transplantation, including ion channel distributions and their development patterns, would help identify the proper EP phenotype of SPC-CMs for future cell therapies.

Transmural and Regional Dispersion of Ventricular Repolarization

Intrinsic transmural and regional electrical heterogeneity in VMs has been demonstrated in human hearts. Based on transmural locations, VMs in large animals display different APDs and compositions of ion channels.[72] For example, compared to VMs located within the

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endo-myocardial layer, epi-myocardial VMs depict larger IK_{to} (transient outward K^+ current) with shorter APDs. Differences in EP properties of these myocardial cells create transmural dispersion of repolarization (TDR) and their differential responses to pharmacologic agents, heart rate changes, neural inputs, and myocardial injury could increase TDR, which has been linked to arrhythmogenesis in genetic disorders, e.g. long QT and Brugada syndromes. [72] In failing hearts, decreased coupling between subepi- and mid-myocardium also increases APD dispersion and may facilitate arrhythmia.[73]

Furthermore, levels and activities of K^+ channel subtypes responsible for various phases of repolarization, such as IK_{to}, ultra-rapid (IK_{UR}), delayed-rectifier (IK_S and IK_r) and inward-rectifier (IK_1) currents, undergo significant changes during embryonic [74] and postnatal [75] development, yet regulation of their developmental changes is not well elucidated. Given that alterations in these K^+ currents are responsible for regional and transmural heterogeneity of ventricular repolarization, unselective transplantation of immature SPC-CMs into undefined layers or regions of myocardium would be expected to increase local or regional TDR and might promote arrhythmia. [71] Thus, the intrinsic transmural and regional electrical heterogeneity of human hearts poses particular problems for cell-based therapies if myocardial repair becomes a reality and a substantial number of transplanted SPC-CMs survive. Furthermore, a greater understanding of the developmental changes of K^+ channels in implanted cells will help in developing electrophysiologically compatible SPC-CMs for cell therapies.

Persistent Automaticity of SPC-CMs

SPC-CMs are a heterogeneous population of cells with variable APDs. Most SPC-CMs display significant phase-4 depolarization with spontaneous automaticity (Table 1). In fact, human ESC-CMs after implantation can form a pulse-generating focus with functional connections to host myocytes and have been used to develop so-called biological pacemakers. [29] These studies demonstrated that these implanted SPC-CMs could easily become automatic foci for frequent premature ventricular complexes (PVCs) observed in most clinical trials of cell therapies. Furthermore, Purkinje-like cells with automaticity have been derived from SPCs in culture (Table 1). Purkinje cells could be the prevalent trigger foci for ventricular arrhythmias or contribute to reentrant circuits in post-infarct VTs. [76] These reports raise the concern that persistent automaticity of SPC-CMs could be an unwanted result of cell therapies.

Immature Intracellular Calcium Handling Capability

Intracellular calcium homeostasis is important for optimal cell function and EC coupling. Imbalance of the intracellular Ca^{2+} handling ability through disease processes (heart failure and ischemia/reperfusion) or drugs can lead to electrical alternans [77] and delayed after-depolarization (DAD), which might induce triggered activity. DAD is the main mechanism for right ventricular outflow tract tachycardia and triggering foci located in Purkinje fibers. During mouse embryonic and postnatal development, expression of ryanodine receptor (RyR2), sarcoplasmic reticulum (SR) Ca^{2+} pump (SERCA2), phospholamban (PLB) and L-type Ca^{2+} channel currents (ICa_L) increases with cardiac maturation yet Na^+/Ca^{2+} exchanger levels remain constant or decrease slightly. [78] In embryonic CMs, Ca^{2+} influx through Ca^{2+} channels is the main source for EC coupling. The SR matures postnatally and plays a major role in EC coupling through the Ca^{2+}–induced Ca^{2+} release apparatus. [79] In human ESC-CMs, these Ca^{2+} handling receptors have been shown to be functionally or developmentally immature. [35, 80] Dysfunctional Ca^{2+} handling by defective RyR2 and calsequestrin has been linked to exercise-induced sudden death in catecholaminergic polymorphic VT and triggered arrhythmia in patients with heart failure. [81] Reports in recent literature are just beginning to study Ca^{2+} handling capabilities of various SPC-CMs and

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might offer insights towards ways to enhance the maturation of intracellular Ca^{2+} handling apparatus of SPC-CMs.

Uncontrolled Sympathetic Hyperinnervation Induced by Cell Transplantation

Sympathetic nerve sprouting has been suggested to be one of the mechanisms of arrhythmogenesis (VF and AF). [82] Direct injection of mesenchymal stem cells into the infarct zone increases sympathetic nerve density throughout the ventricle. [83] Taken together, these results suggest the possibility that cell therapies may induce heterogeneous and unpredictable sympathetic hyper-innervation, which could facilitate cardiac arrhythmia. In addition, the magnitude of SPC-CM responses to adrenergic and cholinergic stimuli has not yet been compared to host myocytes. For instance, L-type Ca^{2+} channels undergo developmental changes in their degree of responses to beta-adrenergic modulation. [84] If these SPC-CMs respond to neurohormonal stimuli differently from host myocardium, implanted cells might become arrhythmogenic during emotional stress or exercise.

Arrhythmogenic Potential Related to the Injection or Transplant Sites

Injected SPCs usually form islands or clusters of cells with questionable or imperfect coupling to host myocardium. [32, 50] Such islands of implanted cells may generate abnormal automaticity and increase dispersion of conduction and refractoriness. In a dog MI model, SkM transplantation caused slow impulse propagation at sites of injection. [85] In a rabbit MI model, injection of SkM in the infarct border zone led to erratic ventricular ectopy, [86] and injection of SkMs in the scar center elicited negative LV remodeling and late ventricular ectopies. [87] Intramyocardial injection of bone marrow mononuclear cells (BMMNCs) in a rat chronic heart failure model [88] also generated cell clusters in the infarct border zone, which most likely were responsible for the increased ventricular arrhythmias. Myocardial injection per se may also lead to scarring. Compared to myocardial injection, intravenous or intracoronary delivery of SPCs seems to be relatively safe in this regard. However, in a swine acute MI model, intravenous delivery of MSCs resulted in shortened effective refractory periods (ERPs) and increased slope of ERP restitution, which would facilitate ventricular arrhythmias. [89] Moreover, implanted SPCs (especially MSCs) could also generate fibroblasts with unknown effects on arrhythmogenesis. Therefore, although implanted SPCs might provide a range of beneficial effects, we must remain cognizant that they could induce electrical heterogeneity at the implant site and lead to arrhythmias.

Arrhythmic Potentials of immature SPC-CMs in Animal Models and Cultures

In animal models and cultured cells, a number of studies have suggested the pro-arrhythmic potential of cell-based therapy. In cell culture models, cultures of human and murine ESC-CMs exhibited heterogeneous EP phenotypes, immature APDs, immature gap junction distributions and spontaneous automaticity, which predispose these ESC-CMs towards propagation failure and triggered arrhythmias.[26, 37, 53, 90–91] Immature intracellular Ca^{2+} handling machinery of these ESC-CMs further increases the pro-arrhythmic potential of these ESC-CMs. [35, 81] Moreover, co-cultures of ≥ 10% MSCs [27] or human SkMs [38] with neonatal rat VMs (NRVMs) resulted in an arrhythmogenic substrate that was characterized by decreased conduction velocity and easily inducible reentrant arrhythmias.[27] These arrhythmias could be decreased by the genetically induced expression of Cx43. [38] Sheets of SkM-derived myotubes also displayed automaticity and caused fibrillation-like cardiomyocyte contractions through stretch-activated channels in a co-culture model. [39]

In animal models, intravenous MSC therapy after acute MI in a swine model improved EF, shortened ventricular ERP, induced uncontrolled sympathetic nerve sprouting, and increased

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the slope of ERP restitution curves, which may increase susceptibility to ventricular arrhythmias. [83,89] In a rat MI model, transplantation of myoblasts, but not BMMNCs, resulted in increased susceptibility of inducible VTs by PES. [92] Injection of SkMs in dog and infusion of SkMs and BM-MSC in rabbit models slowed local ventricular conduction and increased propagation heterogeneity. [85, 93] The relationship of cell retention sites relative to the scars after SkM transplantation further complicates the issue of arrhythmogenic potential of cell therapies (see above).

An important but rarely performed investigation after cell transplantation in animal models involves isolating SPC-CMs from host hearts for maturation studies. Halbach and colleagues recently used a mouse heart slice preparation to assess the properties and functional integration of implanted mouse fetal cardiomyocytes (FCMs) after cryoinjury. [50] Implanted FCMs in the center of the injured region showed no electrical integration and retained fetal AP phenotypes. Some so-called integrated FCMs formed gap junction connections with surrounding viable host myocardium, but these connections displayed a punctate, neonatal pattern of Cx43 distribution. With the immature pattern of integration, conduction blocks occurred at FCM-host myocardium junctions due to failed impulse propagation. These integrated FCMs also displayed immature EP properties. Because this pro-arrhythmic potential and immature development occurs for FCMs, similar results would be expected to occur for immature SPC-CMs. Of note, another study using the same murine model with FCM transplants demonstrated improved short-term cardiac function. [94] Therefore, arrhythmogenesis might be independent from cardiac contractile improvement conferred by cell therapy. Despite benign safety profiles of recent clinical trials, results from these animal models and cultures provide support that adverse EP outcomes could be present if substantial numbers of SPC-CMs survive after transplantation (Figure 4).

A Brief Review of Arrhythmic Risks in Clinical Trials

Clinical trials of cell therapy demonstrated mixed results regarding safety and efficacy, which have been reviewed elsewhere. [2–5] Early clinical trials of SkM transplantation demonstrated significant ventricular arrhythmias. [6–8] Recent trials with SkM transplants, however, reported lower incidence of ventricular arrhythmias [95–97] (but also see [98]). Mechanisms of cell therapies are complex and the reasons for a decreased incidence of ventricular arrhythmia in recent trials remains to be determined. For instance, prophylactic use of amiodarone may have reduced the incidence of arrhythmias.[95] Among various types of SPC sources, infusion of selective types of BMMNCs seems to have provided modest improvement of cardiac function without serious pro-arrhythmic effects.[99–101] However, the ability of such stem cells to generate cardiomyocytes is limited and thus the ameliorating effect is likely due to other beneficial mechanisms.[2–3] Although other side effects of cell therapy, such as coronary restenosis and calcifications, occurred in a few studies, we will focus only on the discussion of arrhythmogenic potentials.

First, the prevalence of arrhythmic events increases with the severity of heart failure.[102] Trials with SkM transplantations were mostly conducted in patients with left ventricular ejection fraction (LVEF) <36% and ventricular arrhythmias occurred more frequently post procedures. Whether these increased arrhythmic events were a reflection of the severity of underlying diseases or the result of intervention remains debatable. [103] Many recent trials with BMMNC, such as ASTAMI, [104] REPAIR-AMI, [105] were conducted on patients with MI and a LVEF more than 40–45% (reviewed in [99–101]). This latter group of patients displays low incidence of arrhythmia-related events or death, [102] which may not allow a firm conclusion about arrhythmogenic risks due to lack of statistical power of these studies. Concomitant coronary revascularization, bypass-surgery and medications with anti-arrhythmic effects (such as beta blockers, statins, and ACE inhibitors) would further reduce

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the incidence of arrhythmias. [99] Consequently, a much larger number of patients with low arrhythmia-related events might be needed in future trials in order to obtain a firm conclusion that the procedure is safe.

Second, most trials applied electrocardiograms, or 24-hour Holter monitoring to determine arrhythmic events at follow-ups. These simple techniques may not be sufficient to detect arrhythmic events based on recent experience in defining the success of atrial fibrillation ablations. [106] Other monitoring techniques such as event monitors, insertable long-term recorders, microvolt T-wave alternans and invasive EP studies with programmed electrical stimulation (PES) protocols [107] should be included, especially for trials with SkM or ESC transplantations. If defibrillators (ICD) or pacemakers were implanted, the specifics of arrhythmia detection algorithms and types of detected arrhythmias should be reported. Inadequate device programming will underestimate arrhythmic events especially with concomitant use of amiodarone. In addition, lack of electrical therapy delivered from an ICD is not the same as lack of increased arrhythmic events. More detailed evaluation of the electrophysiological and arrhythmic consequences of cell-based therapies will improve future trial designs.

Third, human neonatal CMs take 6–10 years to reach adult form in terms of size, shape and gap junction distributions (see above). The developmental status of myocardial protein expression in most SPCs after transplantation in clinical trials is incompletely characterized. In one rare clinical instance, a postmortem study of a patient who received the BMMNC transplant revealed that pericytes just started expressing myocardial proteins at 11 months after cell therapy. [108] While the significance of this result remains to be established, it may suggest that longer follow-up times after cell transplantation are needed because of the extended time required for SPC-CMs to mature completely.

Fourth, most trials with positive outcomes did not provide evidence of true cardiomyogenesis from surviving SPCs. Even if cardiomyocyte differentiation from SPCs did occur, the frequency would expectedly be very low (discussed in [3]) with unclear EP maturation of these SPC-CMs. Moreover, positive outcomes reported from cell transplantation trials showing modest improvement in EF without increasing arrhythmic events might simply indicate that the beneficial effects of cell therapies are from mechanisms other than cardiomyogenesis. In fact, small numbers of surviving, immature SPC-CMs might explain the safety records of recent trials. If a sufficient number of injected cells were to survive and differentiate into immature cardiomyocytes (>10%, see above), they might be pro-arrhythmic. Therefore, the arrhythmogenic potential of cell therapies may depend on the balance between cell retention and cardiomyogenesis on one hand versus other beneficial mechanisms of donor cells on the other.

Concluding Remarks and Future Perspective

This article is intended to raise awareness of possible pro-arrhythmic consequences, stimulate further mechanistic research, help in designing safer clinical trials, enhance better clinical monitoring, and improve the efficacy of cell-based therapy with the ultimate goal of avoiding arrhythmogenic side effects. Current concerns reside in a lack of complete understanding of cardiac differentiation, specification, and maturation, especially regarding EP properties of SPC-CMs. Most SPC-CMs possess immature and diverse EP phenotypes with undefined ion channel compositions. Molecular and genetic factors that regulate ion channel development are mostly unknown. Also, it is unlikely that ischemic or failing hearts would provide a full cardiomyogenic environment for maturation of injected SPCs, and this is aggravated by the variability of diseased myocardium across patients after myocardial damage. In this regard, it is of utmost importance to develop an efficient means of ensuring

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that a physiologically relevant population of VMs will mature normally and integrate properly in patients' hearts. Since normal maturation takes years to complete, it would also be important to develop methods to accelerate maturation in order to reduce unwanted risks during the period of electrophysiological and structural incompatibility. Furthermore, since paracrine effects are the potential beneficial mechanisms of cell therapy, research to develop therapies with the responsible factors [41, 109] might offer a near-term opportunity to improve cell survival and angiogenesis. Thus, much more carefully designed basic research and clinical trials are needed to provide mechanistic insights into various types of cell therapies before they can be considered safe for widespread application. [110]

To reach the aforementioned goals, we recommend a multi-disciplinary approach for future clinical and animal studies, including a cardiac electrophysiologist with expertise in ventricular arrhythmogenesis and a cardiac developmental biologist, to ensure proper trial design, arrhythmia monitoring and interpretation, as well as proper understanding of myocardial cell differentiation, maturation and integration. Additionally, injected SPCs in any trial should be isolated or evaluated for EP characterization several months to years after cell transplantation. For clinical trials, an EP study (EPS) with PES protocols in ventricles, especially for patients with LVEF <40%, [111] should be performed during transplantation and at follow-up to provide more information regarding EP consequences of these experimental cell therapies. Dobutamine or isoproterenol infusion during follow-up stress tests or EPS should also be considered to monitor triggered activities and exercise-induced arrhythmias from newly formed CMs. An event monitor or an ICD/pacemaker with proper arrhythmia detection settings will be very informative about the frequency and occurrence of ventricular arrhythmias. Microvolt T wave alternans test [112] may provide a further assessment of sudden death risk.

Lastly, it is important to highlight several recent scientific breakthroughs for the readers because they have made cell-based myocardial repair close to reality: Combining genetic/ prosurvival factors with cell-based therapy [41] may improve the efficacy of cell replacement and avoid arrhythmogenic potentials. Induced pluripotent stem cell technology reported recently [113] opens the door for future patient-specific, cell-based therapies. [114] Cardiac tissue engineering also demonstrates promises for developing myocardium and bioartificial hearts *ex vivo* for cell replacement therapies. [115]

Acknowledgments

Due to quickly expanding literature in the field of progenitor/stem cell-based therapies, we could not cover every paper published and we express our sincere regret if we missed any important paper or research work.

Funding Sources: This work was supported by an Early Career Development Award from American College of Cardiology Foundation, Bechtel Trusts & Foundation Grant, and California Institute of Regenerative Medicine (CIRM) Grants (RS1-00171-1 and RT1-01143) to H-S.V.C., and by NIH grants (R37 HL059502 and R01 HL083463), CIRM (RC1-00132-1), Exelixis, Inc., and the Mathers Charitable Foundation to M. M.

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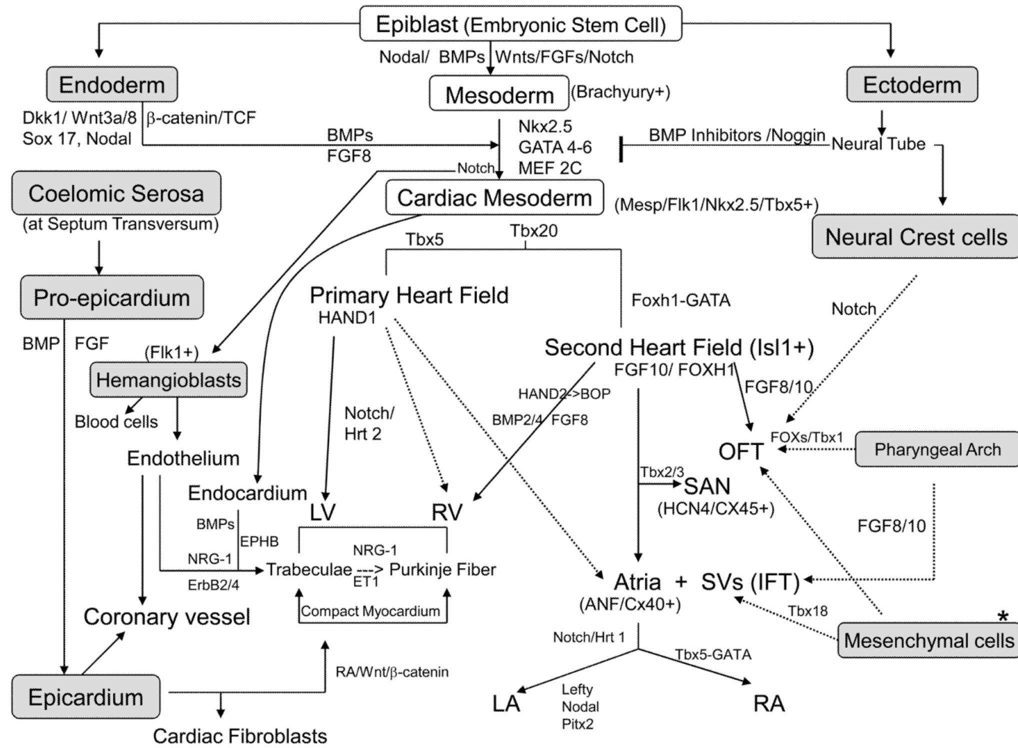

Figure 1.

A simple schematic of molecular pathways of cardiogenesis. See reviews in 9–13 for details. Shaded areas indicate the extra-cardiac cell sources. Dkk1 indicates Dickkopf1; Sox17, an endodermal transcription factor; TCF, T cell factor; BMP, Bone morphogenetic protein; Wnts, Wingless related factors; Nkx, NK family of Homeobox genes; MEF2C, myocyte-specific enhancer-binding factor 2C; Tbx, T-box transcription factors; Flk1, kinase insert domain protein receptor; Isl1: LIM homeodomain transcription factor islet1; FGF, Fibroblast growth factor; EphB, Ephrin-B; PitX, Pituitary homeobox family; NRG-1, neuregulin-1; RA, retinoid acid; ET1, endothelin-1; Hrt, hairy-related transcription factor; BOP, CD8b-opposite transcription factor; Foxh1, forkhead DNA binding transcription factor; Fox, forkhead transcription factor; and * denotes mesenchymal cells from multiple sources.

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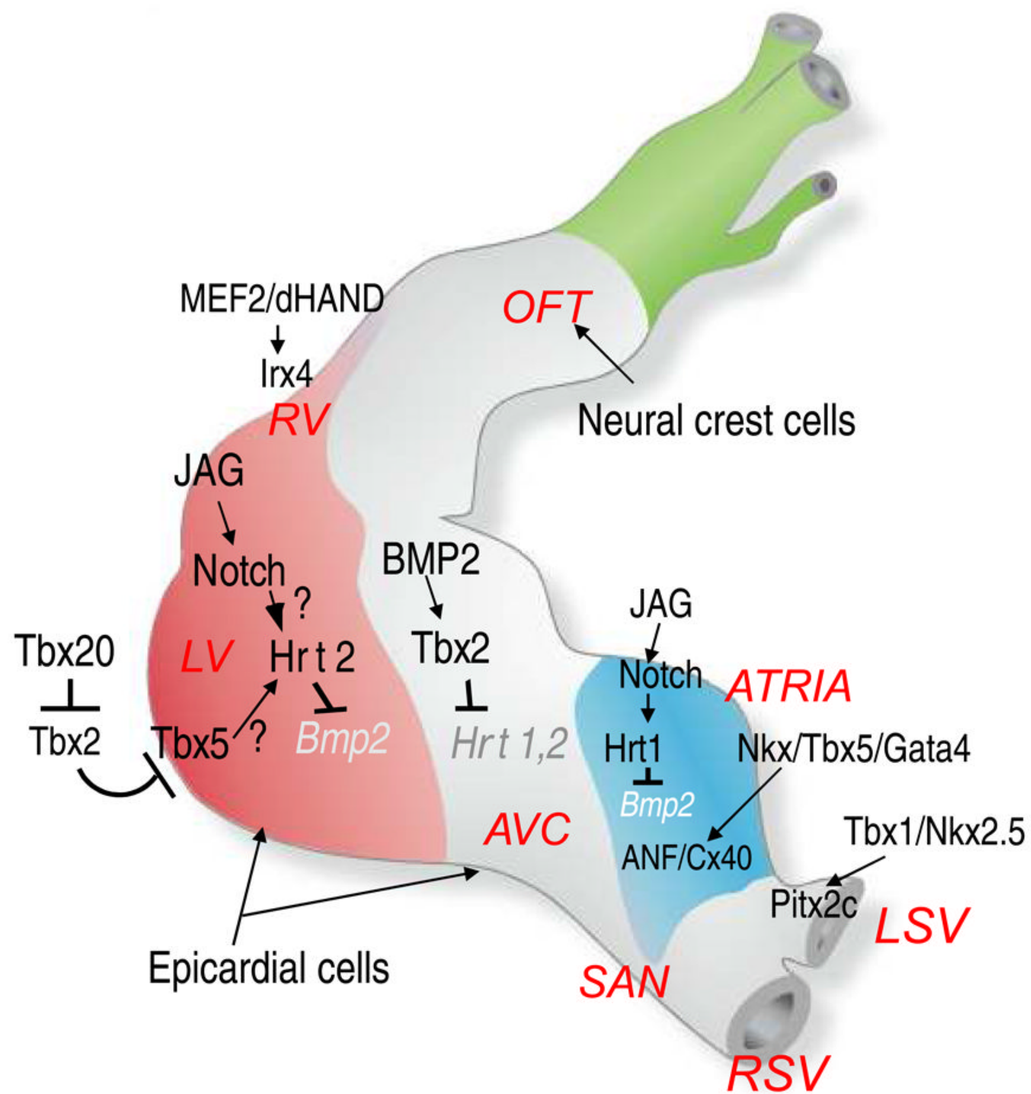

Figure 2.

A diagram of genetic and signaling pathways involved in chamber specification of myocardial development. JAG indicates a notch ligand, *Jagged*; Irx, Iroquois-related homeobox protein; see Figure 1 legend for the definition of other abbreviations. Adapted from reference [18] with permission.

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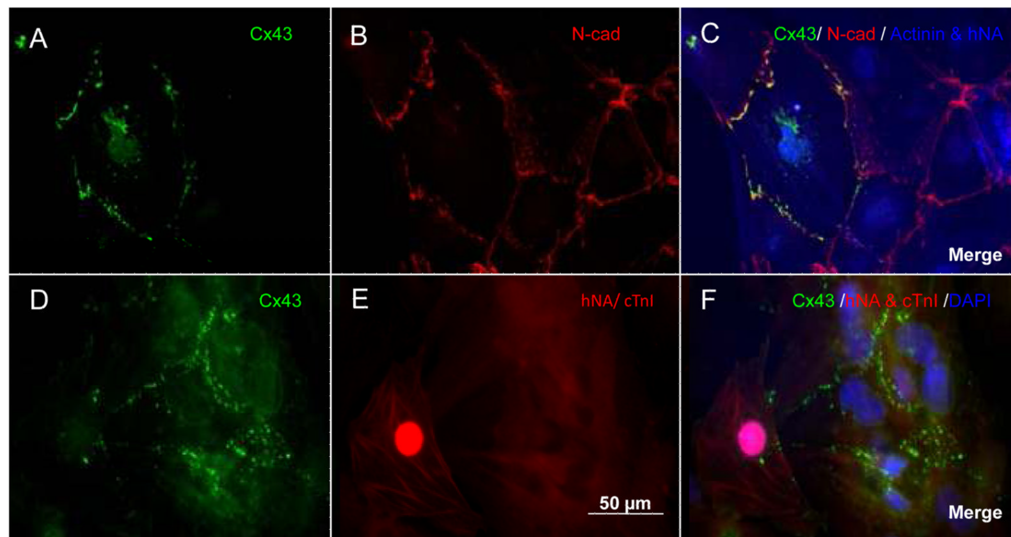

Figure 3.

Expression patterns of connexin 43 (Cx43) in human embryonic stem cell-derived cardiomyocytes (hESC-CMs) and neonatal rat ventricular myocytes (NRVMs). Images are of hESC-CMs in cardiospheres (A–C) and in co-cultures with NRVMs (D–F). hESC-CMs were stained with anti-Cx43 (green) (A), anti-N-cadherin (red) (B), anti-α-actinin (blue), and anti-human nuclear antigen (hNA, blue) (merged in C). In the co-culture of hESC-CMs and NRVMs, myocytes were immunostained with anti-Cx43 (green) (D), anti-hNA and cardiac troponin I (cTnI) (both in red, E), as well as DAPI for nuclear staining (blue) (merged in F). In E, F, only human cells are positive for anti-hNA staining (red). hESC-CMs are variable in size. Cxs 43 of both hESC-CMs and NRVMs distribute in a punctate and neonatal-like pattern at the cell contact surfaces.

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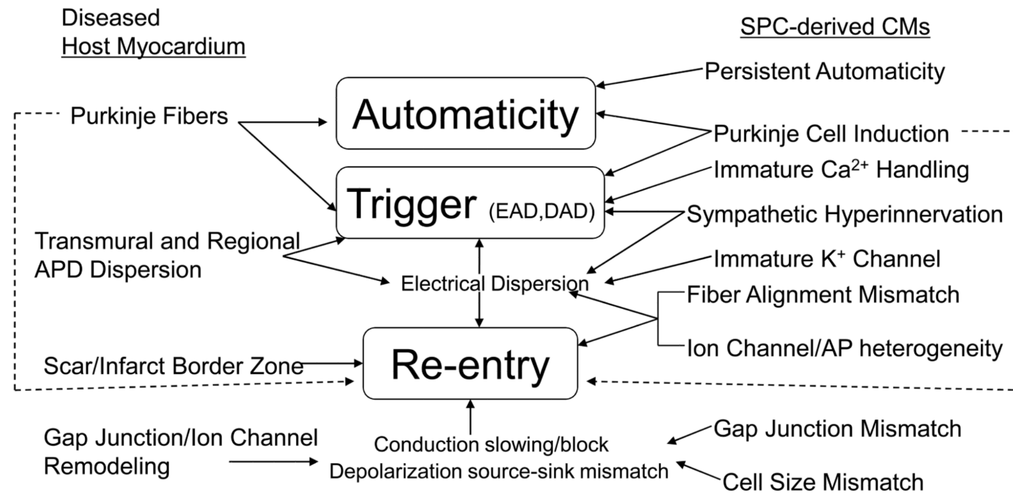

Figure 4.
Summary of potential arrhythmogenic mechanisms of cell-based therapies.

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TABLE 1

Electrophysiological properties of stem and progenitor cell-derived cardiomyocytes.

	Cell Source	HR (bpm)	AP type	MDP (mV)	Vmax (V/s)	Auto.	APD90	APA (mV)	INa	If (HCN)	ICaL	IK1	IK-DR	IKto	B-R	Ach-R	Arrhyth.	Ca Oscil.	CX43	Connect.
Large mammals Adult CM																				
Schram[49]	SAN	70	Nodal/P	−50	<2–15	high	200	70	No	large	strong	min.	IKr,IKs	min.	Y	Y	Physiol.	Y	N	Y
	Atria	min.	A	(−70~−80)	150–300	min.	150	100–110	large	small	mod.	mod.	IKr,IKs	Y	Y	Y	N	N	Y	Y
	V	N	V	−80	200–300	no	200–300	140	strong	none	large	large	IKr,IKs	Y	Y	Y	N	N	Y	Y
	AVN	40–50	AVN	−64	<20	mod.	<120	90	small	large	large	min.	IKr,IKs	Y	Y	Y	Y	Y	N	Y
	Purkinje	<30	Pur	−90	400–800	low	290	130	strong	large	small	large	IKr	Y	Y	?	Y	Y	?	Y
Human fetal CM																				
Mummery[37]	hFetal V	0.8 HZ	fetal V	−38.5	8.9	low	370	69	ND	ND	Y	ND	ND	ND	Y	Y	ND	Y	ND	ND
	hFetal A	1Hz	fetal A	−35	1.2	mod.	164.9	57.2	ND	ND	Y	ND	ND	ND	Y	Y	ND	Y	ND	ND
Murine Fetal CM																				
Halbach[50]	mFetal V	min.	fetel V	−55	<15	min.	125	~60	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	Y	Y
Murine ESC-CM: see reviews[51–52]																				
Human Embryonic SC-CM																				
He[53]	hESC	69	A	−52.6	11.5	Y	131	78.5	ND	ND	ND	ND	IKr	ND	Y	ND	EAD,DAD	ND	ND	ND
		47	V	−53.9	13.2	Y	247	85.3									Y(Irreg.)			
		70	Nodal	−49.2	6.9	Y	168	68.5												
Mummery[37]	hESC	90	A	−38.7	8.5	Y	121.8	60.8	ND	ND	Y	ND	ND	ND	Y	Y	ND	Y	ND	ND
		36	V	−48	7	Y	436.4	80												
		72	P	−20.8	2.6	Y	134	32												
Xu[54]	hESC	40–50	ND	ND	ND	Y	ND	ND	ND	ND	Y	ND	ND	ND	Y	ND	ND	Y	ND	ND
Satin[55]	hESC	54–94	P, V	~−60	12	Y	~450	100	Y	Y	Y	N	ND	ND	Y	Y	ND	Y	Y	Y
Santiani[56]	hESC	26–36	A & V	~−70	4.2–6.0	Y	200–341	80–100	ND	Y	Y	Y	IKr	Y	Y	ND	ND	ND	ND	ND
Mesenchymal SC-CM																				
Takeda[57]	hBM-MSC	66–70	V, Nodal	−50	ND	Y	165–346	47–64	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	Y	N
Makino[58]	mBMSC	108–950	V	−59.5	ND	Y	52	71	ND	ND	Y	ND	ND	ND	Y	ND	ND	ND	Y	ND

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	Cell Source	HR (bpm)	AP type	MDP (mV)	Vmax (V/s)	Auto.	APD90	APA (mV)	INa	If (HCN)	ICaL	IK1	IK-DR	IKto	B-R	Ach-R	Arrhyth.	Ca Oscil.	CX43	Connect.
		143–788	Nodal	−55	ND	Y	42	58.5	ND	ND	ND	ND	ND	ND	Y	ND	ND	ND	Y	ND
Nishiyama[28]	hUCB-MSC	36	A or V	−54	ND	Y	186	57	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	Y	N
BMPC (non-Mesenchym.) CM																				
Badorff[59]	hEPC CD34+	?	ND	ND	ND	?	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	Y	Y	ND	Y
Lagostena [60]	mBM c-kit+	NA	NA	(−55~−65)	NA	N	NA	NA	N	ND	N	Subset	Subset	Subset	ND	ND	NA	N	N	N
Rota[61]	mBM c-kit+	ND	?	ND	ND	Y	ND	ND	Y	ND	ND	Y	Y	ND	ND	ND	?	Y	Y	Y
Adipose Derived SC-CM																				
Bai[62]	hASC	28–47	P	ND	ND	Y	ND	ND	ND	ND	<1%	ND	ND	Y	ND	ND	ND	Y	ND	ND
Planat-Bérard[63]	mASC	7Hz	A, V, P	(−40~−60)	1.8	Y	60–300	68	ND	ND	ND	ND	ND	ND	Y	Y	ND	ND	Y	ND
Yamada[64]	mCD29+	167–490	P	−56.5	ND	Y	50	57.5	ND	ND	Y	ND	ND	ND	Y	ND	ND	ND	ND	ND
Adult Spermatogonial SC-CM																				
Guan[65]	mASpSC	48	A, Purkinje	−57	44, 49	Y	162, 287	88, 96	Y	ND	Y	ND	ND	ND	Y	ND	Y(Irreg.)	Y	Y	Y
			V	−54	31.6	Y	314	93												
			P	−38	2.5	Y	227	63												
			Intermed.	−43	2.9	Y	340	74												
Resident CPCs																				
Laugwitz[14]	mIsl1+	1Hz	A, N	~−60	~60	Y	~220	80	ND	ND	ND	ND	Y	ND	Y	ND	ND	Y	ND	Y
Matsuura [66]	mSca-1+	132	ND	ND	ND	~1%	ND	ND	ND	ND	ND	ND	ND	ND	Y	ND	Y(Irreg.)	Y	Y	ND
Smith[67]	h&p c-kit+	0	P, V	−50, −80	ND	N	~400	75, 110	Y	ND	Y	Y	ND	ND	ND	ND	Y(Irreg.)	Y	Y	Y
Goumans[68]	hSca-1+	40	V	−67	39	Y	483	~90	ND	ND	Y	ND	ND	ND	Y	ND	ND	Y	Y	Y
Skeletal Myoblast																				
Leobon[32]	rSkM	ND	Skeletal	ND	ND	rare	1–5.6	ND	ND	ND	ND	ND	ND	ND	ND	ND	EAD	ND	ND	N
Winitsky[69]	mSpoc cell	1–8 Hz	Skeletal	−60	fast	High	~5	110–150	Y	ND	Y	ND	ND	ND	Y	ND	Y(Irreg.)	Y	ND	ND
Itabashi[39]	rSkM	2–5Hz	Skeletal	ND	fast	0.3	short	~100	ND	ND	N	ND	ND	ND	ND	Y	Y	Y(Fib.)	N	N
Placental SC-CM																				
Okamoto[70]	hPDEMS	63	P, V	−52	4.5	Y	209	64	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	Y	N

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CM indicates cardiomyocyte; h, human; m, murine; r, rat; p, pig; HR, heart rate; AP, action potential; MDP, maximal diastolic potential; Auto, automaticity; APD90, AP duration at 90% of repolarization; APA, AP amplitude; INa, NA^+ current; If (HCN), pacemaker current mediated by hyperpolarization-activated cyclic nucleotide-gated channel; ICaL, L-type Ca^{2+} current; IK1, inward-rectifier K^+ current; IK-DR, delay-rectifier K^+ current; IKr/IKs, rapid and sustained component of IK-DR; IKto, transient outward K^+ current; B-R, beta-adrenergic response; Ach-R, cholinergic response; Arrhyth., arrhythmic activities; EAD, early after-depolarization; DAD, delayed after-depolarization; CA Oscil., intracellular Ca^{2+} oscillation shown by Ca^{2+} imaging; Cx43, Cx43 by staining; Connect., intercellular connections with host cells shown by dye transfer; SAN, sinoatrial node; A, atria; V, ventricle; AVN, atrioventricular node; Pur., Purkinje cell; P, pacemaker cell; Intermed., intermediate type; min., minimal; mod., moderate; Y, positive result; N, negative result; ND, not determined; NA, not applicable; Irreg., irregular rhythm; ESC, embryonic stem cells; MSC, mesenchymal stem cell; BM, bone-marrow; UCB, umbilical cord blood-derived; BMPC, Bone marrow derived progenitor cell; EPC, Endothelial progenitor cell: ASC, adipose derived stem cell; ASpSC, adult spermatogonial stem cell; CPC, cardiac progenitor cell; Isl1, islet-1; Sca-1, stem cell antigen-1; SkM, Skeletal myoblast; PDEMC: placenta-derived extraembryonic mesodermal cell.