Electrophysiological Challenges of Cell-Based Myocardial Repair

Huei-Sheng Vincent Chen, MD, PhD; Changsung Kim, PhD; Mark Mercola, PhD

Cardiovascular diseases remain the major cause of death in the Western world. 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. We refer readers to several excellent reviews for an overview of cell-based therapies for cardiac diseases. 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 transplantation, 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 nonarrhythmogenic (reviewed in References 2 and 4); however, the negligible production of new cardiomyocytes documented for many SPCs employed makes it difficult to extrapolate their safety records toward future trials in which substantial numbers of new cardiomyocytes 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 cardiomyocytes 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 cardiomyocytes exhibit variable electrophysiological properties and are typically immature compared with adult cardiomyocytes (see below). This immaturity of primitive SPC-derived cardiomyocytes remains a major hurdle toward 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-derived cardiomyocytes, that need to be resolved to improve the maturation of primitive SPC-derived cardiomyocytes and to avoid unwanted risks of increasing arrhythmic incidence (proarrhythmia) after transplantation.

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 References 9 to 13). Cardiomyocytes develop from cardiogenic mesoderm (Nkx2.5+ and Mesp1/2+) during embryonic development. In addition to the primary cardiac mesoderm, other sources of cells 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. Cardiomyocytes in the developing fetal heart continue to proliferate, despite functional contraction, but mitotic activity declines quickly after birth (few weeks in rat), and continued growth of the heart is largely due to an increase in myocyte size. In humans, cardiomyocytes reach adult size at ages between 10 and 20 years. 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. 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 extracardiac cells, such as pharyngeal mesodermal cells, mesenchymal cells at arterial and venous poles, and possibly circulating hematopoietic and/or endothelial progenitor cells, 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 proepicardium. 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 cardiomyocytes, 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 because the adult heart seems unlikely to produce the complex array of signaling molecules needed to direct cardiogenic differentiation of uncommitted SPCs. Embryonic stem cells (ESCs) are the extreme case in that they are derived from and resemble cells of preimplantation embryos, form teratomas on transplantation, and appear to require a recapitulation of the cardiogenic differentiation program that occurs in the developing embryo to yield cardiomyocytes.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 factors21 capable of stimulating clinically meaningful differentiation of extracardiac SPCs, such as hematopoietic progenitor cells, also remains controversial.3 Studies reporting induced differentiation of extracardiac SPCs by juxtaposition with adult myocardium in vitro or after implantation in vivo have largely relied on colocalizing 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 extracardiac...
SPCs into cardiomyocytes is a matter of considerable dispute. Therefore, when the broader picture is considered, 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 cells such as ESCs. Because late-stage differentiation to a fully matured ventricular cardiomyocyte population has not been achieved (see below), and these cells are probably too fragile to survive implantation, it would seem that immature cardiomyocytes or committed progenitors offer the most promise. However, the ability of adult myocardium to support further development, maturation, and electrophysiological subtype specification of such cells remains poorly probed and in all likelihood will need to be studied further to elucidate potential means of posttransplant augmentation to avoid unwanted risks.

**Future Electrophysiological Challenges in Cardiomyogenesis**

**Gap Junction Distribution Mismatch**

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

More importantly, ion channels, electric dispersion, and tissue architecture, including fibroblasts, all contribute to normal and abnormal cardiac conduction. Therefore, it is...
necessary to examine many other cellular and tissue attributes to avoid an oversimplified view of cardiac conduction and to provide a true evaluation of functional integration of SPC-derived cardiomyocytes 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 \( (V_{\text{max}} = \text{dV/d}t_{\text{max}}) \). Adult ventricular cardiomyocytes have a cylindrical and elongated shape, contracting along a single axis with a positive force-frequency relationship. Neonatal cardiomyocytes and pacemaker cells, however, have a fusiform or spindle-like appearance. In comparison, most SPCs and SPC-derived cardiomyocytes used for clinical trials or animal models display irregular cell shapes (neonatal-like; Figure 3) with their mean size \((<50 \text{ to } 60 \, \mu\text{m})\) smaller than adult ventricular cardiomyocytes. In contrast, skeletal myoblast–derived myotubes are larger than ventricular cardiomyocytes and display fusiform shapes with characteristics of both skeletal and cardiac cells. Some SPCs (eg, hematopoietic stem cells) may not have significant potential for regenerating myocytes but survive in the damaged heart and contribute to other cell types. Therefore, in addition to immature connexin 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. Size match and cellular alignment of engrafted cells are rarely achieved in most reports; therefore, translational research into methods for stimulating maturation, alignment, and electric coupling of regenerated cardiomyocytes will be required.

**Macroscopic Myocardial Fiber Alignment Mismatch**

Macroscopically, the left ventricular muscle fibers can be separated into 3 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 3 fiber layers work in a coordinate fashion with the supporting fibrous matrix to create a twisting motion for generating efficient cardiac ejection. The alignment of the injected SPC-derived cardiomyocytes with the host muscle fibers has been shown to be discordant or, in rare occasions, concordant. 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 electric dispersion.

**AP Mismatch**

Ion channels are important for excitation-contraction coupling, pulse generation, signal transduction, and cell differentiation/proliferation/maturity. 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 circuits. The AP dura-
tion (APD) in human hearts during the first few years of life increases by \( \approx 20\% \) despite a 16-fold increase in heart weight and a 2.4-fold increase in myocardial cell size.\textsuperscript{15,16,48} Adult ventricular cardiomyocytes are usually quiescent (although excitable) and are characterized by hyperpolarized maximal diastolic potential, relatively long APD, and the absence of spontaneous phase 4 depolarization.\textsuperscript{49}

The Table is a literature search summary of data from electrophysiological characterizations of SPC-derived cardiomyocytes. Compared with electrophysiological properties of adult cardiomyocytes, the Table depicts that most SPC-derived cardiomyocytes, at best, exhibit APs similar to fetal or neonatal cardiomyocytes.\textsuperscript{37,50} Regardless of cell\textsuperscript{51–70} sources, these heterogeneous APs are commonly categorized as sinus node– (or pacemaker–), atrial–, and ventricular-like cardiomyocytes based on overall electrophysiological morphologies.\textsuperscript{53} Implanting such immature SPC-derived cardiomyocytes with variable APDs relative to aging adult cardiomyocytes may not be beneficial.\textsuperscript{71} Furthermore, the ability of hematopoietic progenitor cells or endothelial progenitor cells to form electrophysiologically functional cardiomyocytes by transdifferentiation remains controversial (discussed in Reference 3). Other types of extracardiac SPCs, such as mesenchymal stem cells (MSCs) from bone marrow (BM)\textsuperscript{48} and adipose tissue–derived stem cells,\textsuperscript{63} have been reported to yield heterogeneous populations of cardiomyocytes with various AP shapes (Table), albeit at a very low incidence. In addition, skeletal myoblast–derived myotubes obtained from cultures or after engraftment into myocardium displayed very short APD, fast afterdepolarization, and spontaneous phase 4 depolarization.\textsuperscript{49}

Persistent Automaticity of SPC-Derived Cardiomyocytes

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

Immature Intracellular Calcium Handling Capability

Intracellular calcium homeostasis is important for optimal cell function and excitation-contraction coupling. Imbalance of the intracellular Ca\textsuperscript{2+} handling ability through disease processes (heart failure and ischemia/reperfusion) or drugs can lead to electric alternans\textsuperscript{57} and delayed afterdepolarization, which might induce triggered activity. Delayed afterdepolarization 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 2, sarcoplas-
mic reticulum Ca^{2+} pump, phospholamban, and l-type Ca^{2+} channel currents increases with cardiac maturation, yet Na^+/Ca^{2+} exchanger levels remain constant or decrease slightly.78 In embryonic cardiomyocytes, Ca^{2+} influx through Ca^{2+} channels is the main source for excitation-contraction coupling. The sarcoplasmic reticulum matures postnatally and plays a major role in excitation-contraction coupling through the Ca^{2+}-induced Ca^{2+} release apparatus.79 In human ESC-derived cardiomyocytes, these Ca^{2+} handling receptors have been shown to be functionally or developmentally immature.35,80 Dysfunctional Ca^{2+} handling by defective ryanodine receptor 2 and calsequestrin has been linked to exercise-induced sudden death in catecholaminergic polymorphic ventricular tachycardia and triggered arrhythmia in patients with heart failure.81 Reports in recent literature are just beginning to focus on Ca^{2+} handling capabilities of various SPC-derived cardiomyocytes and might offer insights toward ways to enhance the maturation of intracellular Ca^{2+} handling apparatus of SPC-derived cardiomyocytes.

Uncontrolled Sympathetic Hyperinnervation Induced by Cell Transplantation

Sympathetic nerve sprouting has been suggested to be one of the mechanisms of arrhythmogenesis (ventricular fibrillation and atrial fibrillation).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 hyperinnervation, which could facilitate cardiac arrhythmia. In addition, the magnitude of SPC-derived cardiomyocyte responses to adrenergic and cholinergic stimuli has not yet been compared with host myocytes. For instance, l-type Ca^{2+} channels undergo developmental changes in their degree of responses to β-adrenergic modulation.84 If these SPC-derived cardiomyocytes 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.80,52,50 Such islands of implanted cells may generate abnormal automaticity and increase dispersion of conduction and refractoriness. In a dog MI model, skeletal myoblast transplantation caused slow impulse propagation at sites of injection.85 In a rabbit MI model, injection of skeletal myoblasts in the infarct border zone led to erratic ventricular ectopy,86 and injection of skeletal myoblasts in the scar center elicited negative left ventricular remodeling and late ventricular ectopies.87 Intramyocardial injection of BM mononuclear cells in a rat chronic heart failure model 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 with 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 and increased slope of effective refractory period 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 electric heterogeneity at the implant site and lead to arrhythmias.

Arrhythmic Potentials of Immature SPC-Derived Cardiomyocytes in Animal Models and Cultures

In animal models and cultured cells, a number of studies have suggested the proarrhythmic potential of cell-based therapy. In cell culture models, cultures of human and murine ESC-derived cardiomyocytes exhibited heterogeneous electrophysiological phenotypes, immature APDs, immature gap junction distributions, and spontaneous automaticity, which predispose these ESC-derived cardiomyocytes toward propagation failure and triggered arrhythmias.26,37,53,90,91 Immature intracellular Ca^{2+} handling machinery of these ESC-derived cardiomyocytes further increases the proarrhythmic potential of these ESC-derived cardiomyocytes.35,81 Moreover, cocultures of ≥10% MSCs or human skeletal myoblasts88 with neonatal rat ventricular cardiomyocytes resulted in an arrhythmogenic substrate that was characterized by decreased conduction velocity and easily inducible reentrant arrhythmias.25 These arrhythmias could be decreased by the genetically induced expression of Cx43.39 Sheets of skeletal myoblast–derived myotubes also displayed automaticity and caused fibrillation-like cardiomyocyte contractions through stretch-activated channels in a coculture model.39

In animal models, intravenous MSC therapy after acute MI in a swine model improved ejection fraction, shortened ventricular effective refractory period, induced uncontrolled sympathetic nerve sprouting, and increased the slope of effective refractory period restitution curves, which may increase susceptibility to ventricular arrhythmias.83,89 In a rat MI model, transplantation of myoblasts, but not BM mononuclear cells, resulted in increased susceptibility of inducible ventricular tachycardias by programmed electric stimulation.92 Injection of skeletal myoblasts in dog and infusion of skeletal myoblasts and BM MSCs in rabbit models slowed local ventricular conduction and increased propagation heterogeneity.85,93 The relationship of cell retention sites relative to the scars after skeletal myoblast 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-derived cardiomyocytes 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 after cryoinjury. Implanted fetal cardiomyocytes in the center of the injured region showed no electric integration and retained fetal AP phenotypes. Some so-called integrated fetal cardiomyocytes formed gap junction connections with surrounding...
Table. Electrophysiological Properties of SPC-Derived Cardiomyocytes

<table>
<thead>
<tr>
<th>Cell Source</th>
<th>HR, bpm</th>
<th>AP Type</th>
<th>MDP, mV</th>
<th>Vmax, V/s</th>
<th>Automaticity</th>
<th>APD90</th>
<th>APA, mV</th>
<th>INa</th>
<th>If (HCN)</th>
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<tr>
<td>Schram⁴⁹</td>
<td>SAN</td>
<td>70</td>
<td>Nodal/P</td>
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<td>&lt;15</td>
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<td>200</td>
<td>70</td>
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</tr>
<tr>
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<td>Min</td>
<td>A</td>
<td>(−70 to −80)</td>
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<td>Min</td>
<td>150</td>
<td>100–110</td>
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<td>Small</td>
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<tr>
<td>V</td>
<td>N</td>
<td>V</td>
<td>−80</td>
<td>200–300</td>
<td>No</td>
<td>200–300</td>
<td>140</td>
<td>Strong</td>
<td>None</td>
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<tr>
<td>AVN</td>
<td>40–50</td>
<td>AVN</td>
<td>−64</td>
<td>&lt;20</td>
<td>Mod</td>
<td>&lt;120</td>
<td>90</td>
<td>Small</td>
<td>Large</td>
</tr>
<tr>
<td>Purkinje</td>
<td>&lt;30</td>
<td>Purkinje</td>
<td>−90</td>
<td>400–800</td>
<td>Low</td>
<td>290</td>
<td>130</td>
<td>Strong</td>
<td>Large</td>
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<td>Mummery⁵⁷</td>
<td>hFetal V</td>
<td>0.8 Hz</td>
<td>Fetal V</td>
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<td>8.9</td>
<td>Low</td>
<td>370</td>
<td>69</td>
<td>ND</td>
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<td>hFetal A</td>
<td>1 Hz</td>
<td>Fetal A</td>
<td>−35</td>
<td>1.2</td>
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<td>164.9</td>
<td>57.2</td>
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<td>Strong</td>
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<td>AVN</td>
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<td>AVN</td>
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<td>Mod</td>
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<td>Purkinje</td>
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<td>Halbach⁵⁰</td>
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<td>Fetal V</td>
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<td>He⁵³</td>
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<td></td>
<td>47</td>
<td>V</td>
<td>−53.9</td>
<td>13.2</td>
<td>Y</td>
<td>247</td>
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<td>Xu⁵⁴</td>
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<td>P, V</td>
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<tr>
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<td>hBM-MSC</td>
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<td>V, Nodal</td>
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<td>52</td>
<td>71</td>
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<td>hUCB-MSC</td>
<td>36</td>
<td>A or V</td>
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<td>Y</td>
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<tr>
<td>Badort²⁹</td>
<td>hEPC CD34⁺</td>
<td>slow</td>
<td>ND</td>
<td>ND</td>
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<td>Y</td>
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<td>Lagostena³⁰</td>
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<td>NA</td>
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<td>mBM C-kil⁻</td>
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<td>mASC</td>
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<td>A, V, P</td>
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<td>1.8</td>
<td>Y</td>
<td>60–300</td>
<td>68</td>
<td>ND</td>
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<td>Yamada³⁴</td>
<td>mCD29⁺</td>
<td>167–490</td>
<td>P</td>
<td>−56.5</td>
<td>ND</td>
<td>Y</td>
<td>50</td>
<td>57.5</td>
<td>ND</td>
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<td>Guan³⁵</td>
<td>mAspSC</td>
<td>48</td>
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<td>Y</td>
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<td>Laugwitz³⁶</td>
<td>mlsl1⁺</td>
<td>1 Hz</td>
<td>A, N</td>
<td>(−60 to −60)</td>
<td>~220</td>
<td>80</td>
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<td>Matsaura³⁷</td>
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<td>132</td>
<td>ND</td>
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<td>Smithr³⁷</td>
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<td>P, V</td>
<td>(−50 to −80)</td>
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<td>N</td>
<td>~400</td>
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<td>hSca-1⁺</td>
<td>40</td>
<td>V</td>
<td>−67</td>
<td>39</td>
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<td>483</td>
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<td>Leobon³²</td>
<td>rSkM</td>
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<td>Skeletal</td>
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<td>Okamoto⁴⁰</td>
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CM indicates cardiomyocyte; h, human; m, murine; r, rat; p, porcine; HR, heart rate; AP, action potential; MDP, maximal diastolic potential; APD90, AP duration at 90% of repolarization; APA, AP amplitude; Na⁺, Na⁺ current; If (HCN), pacemaker current mediated by hyperpolarization-activated cyclic nucleotide-gated channel; ICaL, L-type Ca²⁺ 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; EAD, early after-depolarization; DAD, delayed after-depolarization; Ca²⁺ Oscillation, intracellular Ca²⁺ oscillation shown by Ca²⁺ imaging; Cx43, Cx43 staining; Connect., intercellular connections with host cells shown by dye transfer; SAN, sinoatrial node; A, atrium; V, ventricle; AVN, atrioventricular node; Purkinje,
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<td>Y</td>
<td>ND</td>
<td>Y (irregular rhythm)</td>
<td>Y</td>
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Purkinje cell; P, pacemaker cell; Intermed, intermediate type; min., minimal; mod., moderate; Y, positive result; N, negative result; ND, not determined; NA, not applicable; Physiol, physiological variations; UC8, umbilical cord blood-derived; BMSC, Bone marrow derived stromal 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; Spoc, skeletal-based precursors of cardiomyocytes; and PDEMC, placenta-derived extraembryonic mesodermal cell.
viable host myocardium, but these connections displayed a punctate, neonatal pattern of Cx43 distribution. With the immature pattern of integration, conduction blocks occurred at fetal cardiomyocyte–host myocardium junctions because of failed impulse propagation. These integrated fetal cardiomyocytes also displayed immature electrophysiological properties. Because this proarrhythmic potential and immature development occur for fetal cardiomyocytes, similar results would be expected to occur for immature SPC-derived cardiomyocytes. Of note, another study using the same murine model with fetal cardiomyocyte transplants demonstrated improved short-term cardiac function. 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 electrophysiological outcomes could be present if substantial numbers of SPC-derived cardiomyocytes survive after transplantation (Figure 4).

A Brief Review of Arrhythmic Risks in Clinical Trials

Clinical trials of cell therapy demonstrated mixed results relative to safety and efficacy, which have been reviewed elsewhere.2-5 Early clinical trials of skeletal myoblast transplantation demonstrated significant ventricular arrhythmias,6-8 Recent trials with skeletal myoblast transplants, however, reported lower incidence of ventricular arrhythmias95-97 (but also see Menasché et al98). Mechanisms of cell therapies are complex, and the reasons for a decreased incidence of ventricular arrhythmia in recent trials remain to be determined. For instance, prophylactic use of amiodarone may have reduced the incidence of arrhythmias.9 Among various types of SPC sources, infusion of selective types of BM mononuclear cells seems to have provided modest improvement of cardiac function without serious proarrhythmic 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 calcification, 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 skeletal myoblast transplantations were primarily conducted in patients with left ventricular ejection fraction <36%, and ventricular arrhythmias occurred more frequently after 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 BM mononuclear cells, such as the Autologous Stem-Cell Transplantation in Acute Myocardial Infarction (ASTAMI)104 and the Reinforcement of Enriched Progenitor Cells and Infarct Remodeling in Acute Myocardial Infarction (REPAIR-AMI) trials,105 were conducted on patients with MI and a left ventricular ejection fraction >40% to 45% (reviewed in References 99 to 101). This latter group of patients displays a low incidence of arrhythmia-related events or death,102 which may not allow a firm conclusion about arrhythmogenic risks because of the lack of statistical power of these studies. Concomitant coronary revascularization, bypass surgery, and medications with antiarrhythmic effects (such as β-blockers, statins, and angiotensin-converting enzyme inhibitors) would further reduce the incidence of arrhythmias.99 Consequently, a much larger number of patients with low arrhythmia-related events might be needed in future trials to obtain a firm conclusion that the procedure is safe.

Second, most trials applied ECGs or 24-hour Holter monitoring to determine arrhythmic events at follow-ups. These simple techniques may not be sufficient to detect arrhythmic events on the basis of 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 electrophysiological studies with programmed electric stimulation protocols107 should be included, especially for trials with skeletal myoblast or ESC transplantations. If implantable cardioverter-defibrillators 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 electric therapy delivered from an implantable cardioverter-defibrillator 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 cardiomyocytes take 6 to 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 a rare clinical instance, a postmortem study of a patient who received the BM mononuclear cell transplant revealed that pericytes started expressing myocardial proteins at 11 months after cell therapy.\textsuperscript{108} Although 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-derived cardiomyocytes 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 Murry et al\textsuperscript{3}), with unclear electrophysiological maturation of these SPC-derived cardiomyocytes. Moreover, positive outcomes reported from cell transplantation trials showing modest improvement in ejection fraction 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-derived cardiomyocytes 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 proarrhythmic. Therefore, the arrhythmic 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 proarrhythmic 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 relative to electrophysiological properties of SPC-derived cardiomyocytes. Most SPC-derived cardiomyocytes possess immature and diverse electrophysiological phenotypes with undefined ion channel compositions. Molecular and genetic factors that regulate ion channel development are mostly unknown. In addition, 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 the utmost importance to develop an efficient means of ensuring that a physiologically relevant population of ventricular cardiomyocytes will mature normally and integrate properly in patients’ hearts. Because normal maturation takes years to complete, it would also be important to develop methods to accelerate maturation to reduce unwanted risks during the period of electrophysiological and structural incompatibility. Furthermore, because paracrine effects are the potential beneficial mechanisms of cell therapy, research to develop therapies with the responsible factors\textsuperscript{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.\textsuperscript{110}

To reach the aforementioned goals, we recommend a multidisciplinary 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 electrophysiological characterization several months to years after cell transplantation. For clinical trials, an electrophysiological study with programmed electric stimulation protocols in ventricles, especially for patients with left ventricular ejection fraction <40%,\textsuperscript{111} should be performed during transplantation and at follow-up to provide more information on electrophysiological consequences of these experimental cell therapies. Dobutamine or isoproterenol infusion during follow-up stress tests or electrophysiological studies should also be considered to monitor triggered activities and exercise-induced arrhythmias from newly formed cardiomyocytes. An event monitor or an implantable cardioverter-defibrillators/pacemaker with proper arrhythmia detection settings will be very informative about the frequency and occurrence of ventricular arrhythmias. The microvolt T-wave alternans test\textsuperscript{112} may provide a further assessment of sudden death risk.

Finally, 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\textsuperscript{41} may improve the efficacy of cell replacement and avoid arrhythmogenic potentials. Induced pluripotent stem cell technology reported recently\textsuperscript{113} opens the door for future patient-specific, cell-based therapies.\textsuperscript{114} Cardiac tissue engineering also demonstrates promises for developing myocardium and bioartificial hearts ex vivo for cell replacement therapies.\textsuperscript{115}

Acknowledgments

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

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References


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