HHS Public Access

Author manuscript

Card Electrophysiol Clin. Author manuscript; available in PMC 2016 June 01.

Published in final edited form as:

Card Electrophysiol Clin. 2015 June ; 7(2): 357–370. doi:10.1016/j.ccep.2015.03.012.

Arrhythmia in Stem Cell Transplantation

Shone O. Almeida[*], **Rhys J. Skelton**[*,†], **Sasikanth Adigopula**[*], and **Reza Ardehali**[*,‡]

[*] Department of Medicine, Division of Cardiology, David Geffen School of Medicine, University of California, Los Angeles, California 90095

[†] Murdoch Childrens Research Institute, The Royal Children's Hospital, Parkville, Victoria, 3052, Australia

[‡] Eli and Edyth Broad Center for Regenerative Medicine and Stem Cell Research, University of California, Los Angeles, California 90095

Synopsis

Stem cell regenerative therapies hold promise for treating diseases across the spectrum of medicine. Recent clinical trials have confirmed the safety of stem cell delivery to the heart with promising but variable results. While significant progress has been made in the preclinical stages, the clinical application of cardiac cell therapy is limited by technical challenges, including inability to isolate a pure population of cardiac-specific progenitors capable of robust engraftment and regeneration, lack of appropriate pre-clinical animal models, uncertainty about the best mode of delivery, paucity of adequate imaging modalities, and lack of knowledge about the fate of transplanted cells. The inability of transplanted cells to structurally and functionally integrate into the host myocardium may pose arrhythmogenic risk to patients. This is in part dependent on the type of cell transplanted, where the expression of gap junctions such as connexin-43 is essential not only for electromechanical integration, but has also been found to be protective against electrical instability post-transplant. Additionally, certain methods of cell delivery, such as intramyocardial injection, carry a higher rate of arrhythmias. Other potential contributors to the arrhythmogenicity of cell transplantation include re-entrant pathways due to heterogeneity in conduction velocities between graft and host as well as graft automaticity. In this paper, we discuss the arrhythmogenic potential of cell delivery to the heart.

Keywords

Arrhythmia; Stem Cell; Coupling; Cardiomyocyte; Regeneration; Paracrine; Electromechanical

Corresponding Author Dr.Reza Ardehali, Department of Medicine, Division of Cardiology, David Geffen School of Medicine Eli and Edyth Broad Center for Regenerative Medicine and Stem Cell Research, University of California, Los Angeles, 675 Charles E Young Drive South, MRL room 3780, Los Angeles, California 90095, Rardehali@mednet.ucla.edu.

The other authors have nothing to disclose.

Introduction

The human heart has limited regenerative capacity and there is an unmet demand for improved therapies for cardiovascular disease. Both adult stem cells (ASCs) and human pluripotent stem cells (hPSCs) have the potential to facilitate development of cell-based therapies. ASCs have been employed in clinical trials[1,2], and hPSCs have been used extensively to regenerate injured mammalian hearts, including a recent report of non-human primates[3]. However, full clinical translation of stem cell-based therapies has been limited by numerous challenges including the proarrhythmic nature of stem cell derived cardiac grafts. The potential arrhythmic risk may be attributed to differences in electrophysiological maturity[4,5,6], gap junction isotypes, cell orientation and wave propagation between graft and the host myocardium. *In vivo*, the normal myocardial architecture has a unique three-dimensional extracellular matrix, offering cyclic mechanical stress (from rhythmic heart beating), electric stimulation, cell-cell signaling and topographical cues among the cardiomyocytes (CM). Upon injury, the normal architecture is disrupted and CMs are replaced by scar tissue and proliferating fibroblasts, which in turn results in compromise of the heart's structural integrity and adverse remodeling. These structural changes cause anisotrophy, which provides substrates for reentrant arrhythmias. Additionally, the action potential duration prolongation may potentially produce early afterdelpoarizations, or delayed afterdepolarizations. Any attempt to introduce exogenous cells for regenerative purposes should take into consideration the hostile environment, the lack of normal myocardial structure and the potential for the introduction of cells in a microenvironment where normal cardiomyocyte fibers are replaced by scar. The electromechanical integration of the transplanted cells into such an environment may be a farfetched reality, but warrants critical analysis and intense research.

In the following sections, we will discuss candidates for stem cell therapies, the mechanisms of stem cell cardiac graft induced arrhythmogenicity and the requirements for successful integration and electrophysiological coupling of the hPSC cardiac graft to the damaged heart.

Candidates for Cardiac Repair

There are two schools of thought regarding cell therapy for cardiac regeneration: i) delivery of cells into the heart with the goal of survival, maturation, and integration of the transplanted cells for regeneration and replacement of the scar tissue, and ii) delivery of therapeutic cells into the heart, where cells may not survive to physically replace the damaged tissue, but will ultimately lead to regeneration via paracrine effect and recruitment of endogenous cells to repair the scar. While both scenarios could introduce arrhythmia, survival and engraftment of transplanted cells may dangerously serve as a nidus for arrhythmias.

Potential cell candidates to replace cardiomyocytes in the injured heart must generate an action potential, couple this electrical stimulus to contraction and form the necessary gap junctions for action potential propagation and integration with host myocytes[7]. A variety of cell types have been studied as potential candidates for cardiac regeneration **(Table 1)**. Properties such as propensity for electromechanical integration, arrhythmogenicity and risk

of teratoma formation are important considerations in selecting the appropriate cell. Cell sources for cardiac cell therapy include skeletal myoblasts, bone marrow progenitors, resident cardiac stem cells, human embryonic stem cell (hESCs) and induced pluripotent stem cells (iPSCs)[7,8,9]. Human ESCs, iPSCs and resident cardiac progenitor cells have all been reported to differentiate into cardiomyocytes in both *in vivo* and *in vitro* studies, whereas bone marrow Mesenchymal Stem Cells (MSCs) and skeletal myoblasts rely on transdifferentiation[10].

In addition to selecting the appropriate cell candidate for transplantation, other concerns include the quantity of transplanted cells needed to achieve a clinically reasonable graft size, potential for proliferation *in vivo* and the degree of cell retention[7]. Methods for transplantation include intracoronary and direct intramyocardial via a surgical or catheter-based approach[11]. The degree of cell retention is largely dependent on the method of transplantation, whereas cell viability and survival after transplantation also depends on the cell type and the microenvironment. Widimsky et al. reported that after intracoronary injection of bone marrow cells into large animal models and humans, retention rates ranged 1.3-5.3% two hours after transplantation[11]. Various methods of transplantation may also directly influence the arrhythmogenicity of stem cell therapy, as discussed in later sections.

Finally, another aspect important for successful hPSC integration is graft alignment. If not patterned correctly, engrafted cells have a propensity to integrate randomly into the host heart and thereby increasing electric heterogeneity and arrhythmogenic foci. Ultimately, applications such as tissue engineering need to be utilized to ensure optimal graft alignment.

Skeletal Myoblasts—Skeletal Myoblasts (SMs) are a reservoir for skeletal muscle cell regeneration in cases of muscle injury[12,13]. A major source of SMs are satellite cells, resident muscle stem cells responsible for muscle growth, repair and homeostasis[14]. The potential for *in vitro* amplification of satellite stem cells and their ability to self-renew make SMs a desirable target for cardiac stem cell therapy. There are several features unique to skeletal myoblasts. These cells are committed to a myogenic lineage and become functional myocytes regardless, or rather in spite of, environmental cues[12]. Further, SMs continue to proliferate *in vivo* with a high degree of resistance to tissue ischemia, leading to larger graft sizes. In early mice studies, grafts were shown to be viable for as long as three months post-transplantation[15].

Skeletal myoblasts were used in some of the first clinical trials for cardiac regeneration. Despite modest improvements in left ventricular ejection fraction, the increased incidence of sustained ventricular tachycardia in cell-treated patients led to increased concerns regarding cardiac cell therapy[13,16,17]. SMs do not express the gap junctions, *connexin-43* (Cx43) in particular, necessary for electrical coupling with host cardiomyocytes[18–20] discussed in more detail below. Roell and colleagues have shown that large grafts, if uncoupled with host cardiomyocytes, essentially act as a conduction block and thereby serve as a substrate for ventricular arrhythmias[20,21]. Using lentiviral-mediated transduction with Cx43, one study showed that genetically modified SMs had increased electrical stability and decreased arrhythmogenicity[22]. Future research into this approach will undoubtedly provide useful information.

Bone Marrow Progenitors—Bone marrow cells (BMCs) have been used extensively as a candidate for cardiac regenerative therapy. Clinical trials using unfractionated BMCs, mononuclear bone marrow cells (BM-MNC), BMC-derived hematopoietic progenitors, and MSCs have reported the safety of these cells, but the clinical benefit has been debated. Several explanations have been suggested, including that endothelial precursors within bone marrow expressing CD34 and CD133, hematopoietic lineage markers, induce formation of new blood vessels within the infarct bed as well as proliferation of pre-existing vasculature[23]. Bone marrow-derived cells that express CD133 have been hypothesized in several studies to be the critical cell type involved to cardiac functional recovery[24]. One in particular found that in patients with refractory critical limb ischemia treated with bone marrow cells that include CD133+ cells, there was a strong association with increased endothelial proliferation locally and angiogenesis[25]. Neoangiogenesis within the infarct bed is especially important as prior work has shown that post-infarct, the capillary network within the heart is unable to keep up with increased myocardial demand due to hypertrophy and remodeling, leading to infarct extension and further loss of viable tissue. This is mediated by marrow secreted factors such as Vascular Endothelial Growth Factor (VEGF) and Macrophage Chemoattractant Protein-1 (MCP-1)[26], serving to prevent cell apoptosis, reduce collagen deposition and scar formation as well as improve left ventricular function[23].

The second explanation involves the plasticity of bone marrow-derived cells where it is proposed that these cells may have the potential to generate cardiomyocytes. Although this has been reported as a mechanism by which transplanted BMCs exert their beneficial effect, scientific data supporting transdifferentiation to cardiomyocytes is lacking. Several investigators have shown that *in vitro* and in small animal models, BMC-derived progenitors indeed do give rise new cardiomyocytes in addition to contributing to neoangiogenesis in myocardial infarct models[23,27,28]. Other groups have proposed a third mechanism for improved cardiac function, demonstrating fusion of BMCs with somatic cells in *in vitro* and *in vivo* studies[29]. These fusion cells phenotypically function like the recipient cell. Fusion of bone marrow-derived cells has also been seen with hepatocytes in the liver and neurons in the brain. This phenomenon may potentially explain the generation of cardiomyocytes observed after BMC transplantation[29].

Human clinical trials using bone marrow progenitor cells and MSCs were met with fears over arrhythmogenesis given results of prior work with skeletal myoblasts. However numerous studies have observed no increase in ventricular arrhythmogenicity in MSC and bone marrow progenitor treated patients [30–33]. In fact recent studies have suggested a protective effect from an arrhythmia perspective after MSC transplantation, with one study suggesting reversal of cardiac potassium channel remodeling as a possible mechanism[34]. This may also be the result of poor engraftment, with most cells being cleared or otherwise lost from the host heart, thereby eliminating the chance of these cells acting as an arrhythmogenic substrate[35,36]. Furthermore, it has been postulated that paracrine effects of the MSCs may have a beneficial effect in suppressing the arrhythmogenic substrate. Perin and colleagues demonstrated that endocardial injection of autologous bone marrow mononuclear cells in patients with end-stage ischemic heart disease led to improved perfusion and myocardial contractility[37]. Others showed similar results with intracoronary

delivery of BM mononuclear cells[38], consistent with findings in the TOPCARE-AMI trial which showed significant improvement in global left ventricular ejection fraction and reduced end-systolic volumes[30]. While data from the BMC trials have been encouraging, no study has yet confirmed presence of functioning cardiomyocytes derived from BMCs that have integrated into the host myocardium. Future trials and basic research will shed light on this controversial field.

Resident Cardiac Stem Cells—Historically, the heart has been regarded as a terminally differentiated organ, incapable of regeneration. Cardiac growth was thought to be due to increase in cardiomyocyte size rather than number. However this dogma has been challenged by several recent studies. Taking advantage of Carbon-14 dating technology, researchers have shown that cardiomyocyte renewal does in fact occur, albeit at a slow rate of 1% annually at the age of 25 and decaying over time[39]. Using a “mosaic analysis with double markers” mouse model, a recent study found that post-natal cardiomyocyte generation is a rare occurrence and that this capacity is limited to a small population of cardiomyocytes[40], so called resident cardiac stem cells (CSCs). While some have shown increased cardiomyogenesis post-cardiac infarct and injury[41], this remains controversial. CSCs retain stem-cell like properties including self-renewal and multipotentcy with a myocardial-restricted phenotype[42]. They can give rise to cardiomyocytes, smooth muscle and endothelial cells with the ability to replenish the coronary microcirculation in some cases[43]. This small pool of progenitor cells also take part in myocardial homeostasis, serving to replenish cardiomyocytes post-injury and participating in the remodeling process[43].

Although the existence of resident cardiac stem cells in adult mammalian heart has not been entirely characterized, several populations have been well studied. One such population is the c-kit+/Lin− population that was first described by Beltrami et al. and were shown to give rise to myocytes, smooth muscle, and endothelial cells[44]. Since then, they have gained the intrigue of several groups studying their role in cardiac regeneration. One of the first human trials was SCIPIO, a phase I randomized trial of autologous c-kit+ CSCs in ischemic heart failure[45]. CSCs were isolated from the right atrial appendage, expanded in culture and post-coronary artery bypass grafting, the treatment arm underwent intracoronary CSC infusion. Compared with control, CSC-treated patients showed improvements in ejection fraction and a reduction in infarct size at four months post-infusion. Despite these promising outcomes, challenges such as poor survival and retention of CSCs post-transplantation regardless of delivery method have yet to be overcome[46].

Another rising source of autologous derived cardiomyocytes is cardiospheres (CSps), a term first coined by Messina and colleagues in 2004. CSps are a mixture of various cell types, including resident cardiac stem cells, spontaneously differentiated cardiomyocytes, and even vascular cells[47]. These self-assembling multicellular clusters are obtained from post-natal biopsy specimens and have properties of adult cardiac stem cells[48]. Cardiosphere derived cells (CDCs) have been used in animal studies and clinically with promising results, particularly in the CADUCEUS Trial (CArdiosphere-Derived aUtologous stem CElls to reverse ventricULar dysfunction)[49]. Although primarily designed as a safety trial, preliminary data show that intracoronary infusion of CDCs led to decrease in scar size and improved function of infarcted myocardium without a significant difference in rates of

ventricular arrhythmia between control and treatment arms. This has led to the Allogeneic Heart Stem Cells to Achieve Myocardial Regeneration (ALLSTAR) trial which aims to determine the safety and effectiveness of allogeneic CDCs in decreasing infarct size in patients with myocardial infarction[50].

Human Pluripotent Stem Cells—Human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs), collectively known as human pluripotent stem cells (hPSCs) have the potential to be an unlimited source for a variety of tissue specific cell types. Human PSCs can be efficiently differentiated towards a cardiovascular lineage, hence making them an enticing candidate for cell therapy to regenerate the damaged myocardium. Induced pluripotent stem cells overcome the ethical and social concerns raised with hESCs. Human pluripotent stem cells have the advantage of yielding a variety of phenotypes, including atrial, nodal and ventricular cardiomyocytes. Though recent studies have seen major improvements in the efficiency of cardiac differentiation[51,52], shortcomings persist, including teratoma formation with both iPSCs and hESCs and prolonged time to procure and derive iPSCs[53].

Cardiac cells derived from hPSCs can readily engraft into the injured heart and generate a spontaneous action potential[3]. While this makes hPSCs ideal candidates for cell therapy, it also raises legitimate concerns over their arrhythmogenicity. Several studies have reported that PSC-derived cardiomyocytes exhibit immature and fetal-like electrical activities which would make the electromechanical coupling of these cells with the host cardiomyocytes a challenge[4–6]. Additionally, there still remains a significant challenge in isolating a pure population of chamber-specific cardiomyocytes from an *in vitro* differentiation assay. Generally, hPSC differentiation does not yield 100% purity for cardiomyocytes and moreover the generated myocytes represent a heterogenous population that includes ventricular, atrial and nodal cells. It has been suggested that transplanted hESC-derived cardiomyocytes display after-depolarizations due to a low expression of the iK1 channel[54] and also have pacemaking currents independent of the host myocardium[55,56]. Additionally due to their allogenic origin, they are at risk for host immune rejection[53], a potential mechanism for arrhythmogenicity discussed in more detail below. Finally, as is possible with introduction of any cell type, the transplanted cells may modify the substrate with ectopic electrical activities such that an arrhythmogenic focus is generated.

While the electromechanical coupling of PSC-derived cardiomyocytes in the heart remains a significant concern, the host environment may play an essential role. Ardehali et al. for the first time showed structural and functional integration of hESC-derived cardiovascular progenitors into human fetal hearts[57]. Shiba also demonstrated that hESC derived CMs can electrically couple in guinea pig models and actually suppress arrhythmias in the injured heart, seemingly by forming a ‘conduction bridge’ over the scar tissue[58]. Fully understanding the arrhythmogenicity of hPSC cardiac cell transplants ultimately requires additional large animal studies with precise assessment of electrical activities that are propagated throughout the grafted cells. It is speculated that the proarrhythmic properties of hPSC-derived cardiac cells grafts are due to their immature electrophysiological phenotype and may be avoided by the employment of *in vitro* maturation methods prior to transplantation[59].

Electrophysiological Studies and Cell Coupling

The clinical application of stem cells to replenish new myocytes in the heart relies on electromechanical coupling of the transplanted cells with the host. Also important is the ability of the transplanted cells to generate action potentials and thereby perhaps function as biological pacemakers. This automaticity was studied in *in vitro* models which revealed that hESCs exhibit spontaneous electrical activity though with significant rhythmic variation[60]. Automaticity can be studied *in vitro* using whole-cell voltage clamp and simultaneous patch-clamp/laser scanning confocal calcium imaging[61]. Studies have also shown that the coupling between excitation and contraction is related to calcium-induced calcium release – that is local calcium release from the sarcoplasmic reticulum ('calcium clock') and activation of voltage gated ion channels[60,61]. Disruption in either of these mechanisms leads to dysrhythmic beating or in some cases, suppression of automaticity altogether. Kehat also demonstrated electromechanical coupling *in vitro*[62]. Within 24-hours of co-culturing human embryonic stem cell-derived cardiomyocytes (hESC-CMs) with neonatal rat ventricular myocytes, synchronous mechanical activity was detected. High resolution activation maps that characterize impulse initiation and propagation revealed close temporal coupling between graft and host. Electrophysiological analysis has also shown that hESC-CMs express many of the same ion channels as mature cells[61,62].

Electromechanical integration of hESC-CMs into injured hearts is essential to improving cardiac function **(Figure 1)**. Several *in vivo* studies have elegantly demonstrated that delivery of hESC-CMs into an injured heart leads to at least partial coupling of the transplanted cells with the host cardiomyocytes. One group showed that these cells in fact form new force-generating units[58]. The investigators used genetically modified hESC-CMs that encoded a fluorescent calcium sensor such that post-transplantation, epicardial fluorescent transients could be correlated with electrocardiogram to demonstrate synchrony with host myocardium. Ardehali established that when hESC-derived cardiovascular progenitors are transplanted in human fetal hearts, they are able to migrate and couple with neighboring host cardiomyocytes, exhibiting synchronous electrical activity[57]. Others have also demonstrated that transplanted hESC-CMs survive and integrate *in vivo*[62]. In fact, using a pig complete heart block model, Kehat and colleagues showed that the transplanted cells displayed automaticity and biological pacing functionality.

For functional integration to occur, the electrical potential generated in one cell must be sufficient to propagate through gap junctions and depolarize neighboring cells[62]. Indeed it is the disruption of this structure through loss of desmosomes and gap junctions in ischemic disease that leads to arrhythmia in the injured heart[63]. One gap junction of particular importance is *connexin-43 (Cx43)*[20,57,62,63,64]. It has been shown that transplantation of embryonic cardiomyocytes led to increased electrical stability in the injured heart, particularly improved coupling between graft and host and decreased incidence of ventricular tachycardia, a property that is dependent on connexin-43[20,65]. In fact, transplantation of skeletal myoblasts that do not express Cx43 showed significant increase in the rate of arrhythmias. Similar findings were shown in another study where a hypoxic culture environment served to restore connexin-43 in mesenchymal stem cells, thereby curbing the incidence of arrhythmias[66]. Nevertheless, expression of Cx43 is not in itself

sufficient to suppress the arrhythmic potential of stem cell transplantation and various other mechanisms exist.

In addition to electromechanical coupling and formation of gap junctions, another mechanism that may have confounding effects on the induction of arrhythmia is cell fusion. Studies have shown that bone marrow derived cells selectively fuse with cells in the brain, liver, and heart[29,67]. In sex mismatch studies with transplanted hESC-derived cardiomyocytes to investigate the degree of cell fusion observed, less than 3.8% of transplanted cells showed evidence of fusion, suggesting that fusion events are rare and perhaps transdifferentiation is the dominant process[57]. The key question of whether these fusions have a role in the formation of new cells or a repair and maintenance function remains unanswered.

Mechanisms of Arrhythmogenicity

Various mechanisms have been described for the proarrhythmic potential of stem cell transplantation **(Figure 2)**. In part these mechanisms are largely dependent on the type of cells transplanted as discussed above.

Re-Entrant Pathways and Automaticity—In a study by Liao et al., the proarrhythmic risk of hESCs vs. hESC-CMs was investigated in a mouse model of myocardial infarction (MI)[68]. Through *in vitro* and *in vivo* experimental evidence, the authors revealed increased arrhythmogenesis in the hESC-CM population, particularly prolongedaction potential duration, which led to a higher rate of inducible ventricular tachycardia than the hESC group. One explanation is that the relative difference in action potential duration between transplanted hESC-CMs and intrinsic ventricular CMs facilitates reentrant excitation. Another proposed mechanism is that hESC-CMs can cause abnormal impulse initiations, serving as ectopic arrhythmic foci, early afterdepolarization (EAD), or delayed afterdepolarization (DAD). The *in vivo* experiments demonstrated that while cardiomyocytes integrate with host myocardium, they exhibit immature electrophysiological properties that may lead to less organized gap junctions[65]. These properties predispose the substrate to higher rates of arrhythmia.

The reported degree of electrical instability and arrhythmia rate appears to be quite variable in the literature, however. One possible explanation for the conflicting data may be differences in heart size and rate of the animal models. Many studies have relied on the murine model for *in vivo* cell transplantation studies. However, considering that the intrinsic heart rate in mice is approximately 500-600 beats-per-minute, hPSC-CMs will fail to couple with the mouse cardiomyocytes to maintain such an elevated contraction rate. Using a macaque model of MI, researchers showed that electrical coupling occurs between graft and host myocardium[3]. All transplanted primates demonstrated electromechanical coupling evidenced by epicardial fluorescent calcium transients that were synchronous with host electrocardiogram. However hESC-CM transplanted primates showed arrhythmias, particularly premature ventricular contractions and ventricular tachycardia[3]. This was especially evident in the first two weeks post-transplantation. The coupling rates seen in this large-animal study was higher than seen in experiments by Shiba, where in a guinea-pig MI

model, only 60% of transplanted hearts demonstrated electrical coupling[58]. Interestingly, when transplanted into uninjured hearts, there was 100% electromechanical coupling, suggesting that graft behavior is more heterogenous in injured heart models[58]. Additionally, hESC-CM transplanted guinea pigs showed the lowest fraction of PVCs and spontaneous ventricular tachycardia as well as overall a higher rate of electrical stability in studies evaluating inducible arrhythmias with programmed electrical stimulation[20,58]. This was also seen in similar experiments with mice[69] and rats[70]. Possible mechanisms for observed arrhythmias include the presence of re-entrant circuits as well as graft automaticity[62].

The differences in arrhythmia rate observed in large versus small animals appear to be related, at least in part, to variation in heart size and rate[3]. As mentioned above in murine models, graft integration with host myocytes is immature and with slower rates of ventricular action potential conduction[68]. This phenomenon may be accentuated in large hearts where larger grafts are used, leading to an even slower rate of action potential conduction and predisposing to re-entrant loops[3]. This may explain why increased arrhythmogenicity is seen in larger animal studies rather than with mice and guinea pigs. An alternate explanation surrounds the species-specific heart rate. Faster heart rates as seen in mice (600beats/min) and guinea pigs (230beats/min) favor native conduction pathways over graft automaticity or re-entrant loops[3]. Conversely, macaques have rates between 100-130beats/min. This slower rate may have increased susceptibility to graft automaticity and ventricular arrhythmias.

Impurities in Stem Cell Differentiation—The process of differentiating human embryonic stem cells to cardiomyocytes is an imperfect one. The yield of these protocols is never 100%, with isolates often containing non-cardiac derivatives, and may be contaminated with residual undifferentiated pluripotent stem cells capable of forming teratomas *in vivo*. One explanation for arrhythmogenicity with stem cell transplantation may lie in the impurities of the transplanted graft. This hypothesis was tested using a guinea-pig chronic infarct model[71]. At twenty-eight days post-cardiac cryoinjury, animals were transplanted with hESC-CMs, non-cardiac hESC derivatives or vehicle, the latter two serving as controls. Interestingly there was no statistically significant difference in arrhythmia rate between the three groups outside of the peri-procedural period. All animals then underwent electrophysiological studies to assess the electrical stability. Of the three groups, guinea pigs transplanted with non-cardiac hESC derivatives showed the highest degree of electrical instability with a greater incidence of inducible ventricular tachycardia. The hESC-CM and vehicle groups were fairly arrhythmia resistant. This data suggests that one possible mechanism for arrhythmogenicity in stem cell transplantation is impurity in the cardiomyocyte differentiation process. It is suggested that immunological mechanisms could potentially explain why this leads to higher arrhythmia rates[71]. Transplantation of non-cardiac derivatives could evoke a stronger and more intense host immune response to the graft, leading to increased rejection and thereby increased arrhythmogenicity. However this hypothesis was not supported in follow-up immunohistochemical studies[71]. Several investigators have isolated hESC-derived cardiomyocytes or cardiovascular progenitors using specific surface markers to circumvent the impurity issue[57,72,73]. Identification of

markers that allow for prospective isolation of hESC-derived cardiovascular cells at different stages of development is promising and warrants further investigation.

Confounding Factors—In addition to the mechanisms outlined above, perhaps there are confounding factors in the mechanism of arrhythmogenicity in stem cell transplantation that are in fact cell-independent[74]. These may include local injury or edema induced by myocardial injection[65] as well as variation in transplantation methods. Few head-to-head studies exist comparing delivery methods, but one in particular showed that intramyocardial injection of bone marrow cells was much more arrhythmogenic, including higher rates of ventricular tachycardia, than retrograde intracoronary delivery[75]. One may postulate that injection of cell clusters via the intramyocardial route serves to impede electrical conduction in the host myocardium as well as stimulate cytokine release from inflammatory cells, both of which may lead to higher rates of arrhythmias. It has been also shown that transplantation of mesenchymal stem cells induces nerve sprouting and high sympathetic nerve density[76]. While increased sympathetic innervations could lead to improved contractility and left ventricular ejection fraction, it could also result in higher rates of arrhythmia in myocardium that is already damaged by ischemia[77].

Paracrine Effects

Several studies have evaluated how paracrine effects influence the graft electrical activity. Some suggest that secretion of soluble factors such as cytokines, chemokines, and growth factors from transplanted cells may lead to beneficial effects. This has come to be known as the ‘paracrine hypothesis’[53]. While further work is needed, potential mechanisms for the beneficial effects include the release of cryoprotective molecules that increase native cardiomyocyte survival, neovascularization including angiogenesis and arteriogenesis, alterations in the extracellular matrix resulting in remodeling that leads to increased scar strength and reduced ventricular dilation, improved cardiac contractility, and finally recruitment and activation of resident cardiac stem cells[78]. Some groups have also studied how the *in vitro* environment in which cells are cultured affects their arrhythmic potential. Hwang and colleagues investigated the effects of paracrine media (media conditioned by growing cells) under hypoxic or normoxic conditions[66]. Using myocardial infarct models in rats, they injected hypoxic paracrine media, normoxic paracrine media, or mesenchymal stem cells into the infarct border zone. The hypoxic, but not normoxic, paracrine media was found to prevent sudden death in rats by improving conduction in the border zone through recovery of gap junctions, reducing the degree of fibrosis, and better modulating calcium regulatory ion channels, thereby leading to increased electrical stability.

Conclusion

Research in cardiac regeneration has come a long way. Indeed it has moved from bench to bedside with promising results in human studies. There is still much more to learn though, particularly how to safely use cell therapy to improve conditions such as congestive heart failure and ischemic heart disease while minimizing arrhythmogenicity of cell therapy. Further work is needed to improve methods of cell delivery and transplantation. Newer delivery systems include cell-seeded patches and scaffold-free cell sheets. Cell coupling and

engraftment is also of vital importance to reduce risk of re-entrant pathways and automaticity that serve as a nidus for arrhythmia. From cell selection to proper graft alignment, finding ways to curb the proarrhythmic risk of stem cell transplantation is an essential step towards successful clinical application.

Acknowledgments

Dr. Ardehali is supported by the NIH Director's New Innovator's Award (DP2HL127728) and the California Institute for Regenerative Medicine (RC1-00354-1).

References

1. Bolli R, et al. Effect of Cardiac Stem Cells in Patients with Ischemic Cardiomyopathy: Initial Results of the SCIPIO Trial. Lancet. 2011; 378:1847–1857. [PubMed: 22088800]
2. Menasché P, et al. The Myoblast Autologous Grafting in Ischemic Cardiomyopathy (MAGIC) trial: first randomized placebo-controlled study of myoblast transplantation. Circulation. 2008; 117:1189–1200. [PubMed: 18285565]
3. Chong JJH, et al. Human embryonic-stem-cell-derived cardiomyocytes regenerate non-human primate hearts. Nature. 2014; 510:273–277. [PubMed: 24776797]
4. Mummery C, et al. Differentiation of human embryonic stem cells to cardiomyocytes: role of coculture with visceral endoderm-like cells. Circulation. 2003; 107:2733–2740. [PubMed: 12742992]
5. Laflamme MA, Murry CE. Heart regeneration. Nature. 2011; 473:326–335. [PubMed: 21593865]
6. Snir M, et al. Assessment of the ultrastructural and proliferative properties of human embryonic stem cell-derived cardiomyocytes. Am. J. Physiol. Heart Circ. Physiol. 2003; 285:H2355–2363. [PubMed: 14613910]
7. Schuldt AJT, Rosen MR, Gaudette GR, Cohen IS. Repairing damaged myocardium: evaluating cells used for cardiac regeneration. Curr. Treat. Options Cardiovasc. Med. 2008; 10:59–72. [PubMed: 18325308]
8. Lyon AR, Harding SE, Peters NS. Cardiac stem cell therapy and arrhythmogenicity: prometheus and the arrows of Apollo and Artemis. J Cardiovasc. Transl. Res. 2008; 1:207–216. [PubMed: 20559921]
9. Miyoshi S, et al. Cardiac cell therapy and arrhythmias. Circ. J. Off. J. Jpn. Circ. Soc. 2007; 71(Suppl A):A45–49.
10. Meng X. Transdifferentiation during Heart Regeneration. J. Stem Cell Res. Ther. 2014; 04
11. Widimsky P, et al. Intracoronary transplantation of bone marrow stem cells: background, techniques, and limitations. Eur. Heart J. Suppl. 2006; 8:H16–H22.
12. Oettgen P. Cardiac Stem Cell Therapy Need for Optimization of Efficacy and Safety Monitoring. Circulation. 2006; 114:353–358. [PubMed: 16864740]
13. Siminiak T, Kalmucki P, Kurpisz M. Autologous skeletal myoblasts for myocardial regeneration. J. Intervent. Cardiol. 2004; 17:357–365. [PubMed: 15546287]
14. Scharner J, Zammit PS. The muscle satellite cell at 50: the formative years. Skelet. Muscle. 2011; 1:28. [PubMed: 21849021]
15. Koh GY, Klug MG, Soonpaa MH, Field LJ. Differentiation and long-term survival of C2C12 myoblast grafts in heart. J. Clin. Invest. 1993; 92:1548–1554. [PubMed: 8376605]
16. Menasché P, et al. Autologous skeletal myoblast transplantation for severe postinfarction left ventricular dysfunction. J. Am. Coll. Cardiol. 2003; 41:1078–1083. [PubMed: 12679204]
17. Smits PC, et al. Catheter-based intramyocardial injection of autologous skeletal myoblasts as a primary treatment of ischemic heart failure: clinical experience with six-month follow-up. J. Am. Coll. Cardiol. 2003; 42:2063–2069. [PubMed: 14680727]
18. Reinecke H, MacDonald GH, Hauschka SD, Murry CE. Electromechanical coupling between skeletal and cardiac muscle. Implications for infarct repair. J. Cell Biol. 2000; 149:731–740. [PubMed: 10791985]

19. Gepstein L, et al. In vivo assessment of the electrophysiological integration and arrhythmogenic risk of myocardial cell transplantation strategies. Stem Cells Dayt. Ohio. 2010; 28:2151–2161.
20. Roell W, et al. Engraftment of connexin 43-expressing cells prevents post-infarct arrhythmia. Nature. 2007; 450:819–824. [PubMed: 18064002]
21. Perumal Srinivasan S, et al. Enhanced gap junction expression in myoblast-containing engineered tissue. Biochem. Biophys. Res. Commun. 2012; 422:462–468. [PubMed: 22579687]
22. Abraham MR, et al. Antiarrhythmic engineering of skeletal myoblasts for cardiac transplantation. Circ. Res. 2005; 97:159–167. [PubMed: 15976318]
23. Kocher AA, et al. Neovascularization of ischemic myocardium by human bone-marrow-derived angioblasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function. Nat. Med. 2001; 7:430–436. [PubMed: 11283669]
24. Bartunek J, et al. Intracoronary injection of CD133-positive enriched bone marrow progenitor cells promotes cardiac recovery after recent myocardial infarction: feasibility and safety. Circulation. 2005; 112:I178–183. [PubMed: 16159812]
25. Raval AN, et al. Bilateral administration of autologous CD133+ cells in ambulatory patients with refractory critical limb ischemia: lessons learned from a pilot randomized, double-blind, placebo-controlled trial. Cytotherapy. 2014; 16:1720–1732. [PubMed: 25239491]
26. Fuchs S, et al. Transendocardial delivery of autologous bone marrow enhances collateral perfusion and regional function in pigs with chronic experimental myocardial ischemia. J. Am. Coll. Cardiol. 2001; 37:1726–1732. [PubMed: 11345391]
27. Handgretinger R, Kuçi S. CD133-Positive Hematopoietic Stem Cells: From Biology to Medicine. Adv. Exp. Med. Biol. 2013; 777:99–111. [PubMed: 23161078]
28. Orlic D, et al. Bone marrow stem cells regenerate infarcted myocardium. Pediatr. Transplant. 2003; 7(Suppl 3):86–88. [PubMed: 12603699]
29. Alvarez-Dolado M, et al. Fusion of bone-marrow-derived cells with Purkinje neurons, cardiomyocytes and hepatocytes. Nature. 2003; 425:968–973. [PubMed: 14555960]
30. Assmus B, et al. Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction (TOPCARE-AMI). Circulation. 2002; 106:3009–3017. [PubMed: 12473544]
31. Hare JM, et al. A randomized, double-blind, placebo-controlled, dose-escalation study of intravenous adult human mesenchymal stem cells (prochymal) after acute myocardial infarction. J. Am. Coll. Cardiol. 2009; 54:2277–2286. [PubMed: 19958962]
32. Heldman AW, et al. Transendocardial mesenchymal stem cells and mononuclear bone marrow cells for ischemic cardiomyopathy: the TAC-HFT randomized trial. JAMA. 2014; 311:62–73. [PubMed: 24247587]
33. Hendrikx M, et al. Recovery of regional but not global contractile function by the direct intramyocardial autologous bone marrow transplantation: results from a randomized controlled clinical trial. Circulation. 2006; 114:I101–107. [PubMed: 16820557]
34. Cai B, et al. Bone marrow mesenchymal stem cells protected post-infarcted myocardium against arrhythmias via reversing potassium channels remodelling. J. Cell. Mol. Med. 2014; 18:1407–1416. [PubMed: 24780005]
35. Dai W, Hale SL, Kay GL, Jyrala AJ, Kloner RA. Delivering stem cells to the heart in a collagen matrix reduces relocation of cells to other organs as assessed by nanoparticle technology. Regen. Med. 2009; 4:387–395. [PubMed: 19438314]
36. Hale SL, Dai W, Dow JS, Kloner RA. Mesenchymal stem cell administration at coronary artery reperfusion in the rat by two delivery routes: a quantitative assessment. Life Sci. 2008; 83:511–515. [PubMed: 18755200]
37. Perin EC, et al. Transendocardial, autologous bone marrow cell transplantation for severe, chronic ischemic heart failure. Circulation. 2003; 107:2294–2302. [PubMed: 12707230]
38. Strauer BE, et al. Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. Circulation. 2002; 106:1913–1918. [PubMed: 12370212]
39. Bergmann O, et al. Evidence for Cardiomyocyte Renewal in Humans. Science. 2009; 324:98–102. [PubMed: 19342590]

40. Ali SR, et al. Existing cardiomyocytes generate cardiomyocytes at a low rate after birth in mice. Proc. Natl. Acad. Sci. 2014; 111:8850–8855. [PubMed: 24876275]
41. Senyo SE, et al. Mammalian heart renewal by pre-existing cardiomyocytes. Nature. 2013; 493:433–436. [PubMed: 23222518]
42. Nadal-Ginard B, Anversa P, Kajstura J, Leri A. Cardiac stem cells and myocardial regeneration. Novartis Found. Symp. 2005; 265:142–154. discussion 155–157, 204–211. [PubMed: 16050255]
43. Torella D, Ellison GM, Méndez-Ferrer S, Ibanez B, Nadal-Ginard B. Resident human cardiac stem cells: role in cardiac cellular homeostasis and potential for myocardial regeneration. Nat. Clin. Pract. Cardiovasc. Med. 2006; 3(Suppl 1):S8–13. [PubMed: 16501638]
44. Beltrami AP, et al. Adult cardiac stem cells are multipotent and support myocardial regeneration. Cell. 2003; 114:763–776. [PubMed: 14505575]
45. Chugh AR, et al. Administration of Cardiac Stem Cells in Patients With Ischemic Cardiomyopathy: The SCIPIO Trial Surgical Aspects and Interim Analysis of Myocardial Function and Viability by Magnetic Resonance. Circulation. 2012; 126:S54–S64. [PubMed: 22965994]
46. Hong KU, Bolli R. Cardiac stem cell therapy for cardiac repair. Curr. Treat. Options Cardiovasc. Med. 2014; 16:324. [PubMed: 24903489]
47. Messina E, et al. Isolation and Expansion of Adult Cardiac Stem Cells From Human and Murine Heart. Circ. Res. 2004; 95:911–921. [PubMed: 15472116]
48. Barile L, et al. Human Cardiospheres as a Source of Multipotent Stem and Progenitor Cells. Stem Cells Int. 2013; 2013:e916837.
49. Malliaras K, et al. Intracoronary Cardiosphere-Derived Cells After Myocardial Infarction: Evidence of Therapeutic Regeneration in the Final 1-Year Results of the CADUCEUS Trial (CArdiosphere-Derived aUtologous stem CElls to reverse ventricULar dySfunction). J. Am. Coll. Cardiol. 2014; 63:110–122. [PubMed: 24036024]
50. Capricor Inc. Allogeneic Heart Stem Cells to Achieve Myocardial Regeneration (ALLSTAR). National Library of Medicine (US); Bethesda (MD): 2000- [23 Jan 2015]. Available from https://clinicaltrials.gov/ct2/show/NCT01458405 NLM Identifier: NCT01458405
51. Lian X, et al. Robust cardiomyocyte differentiation from human pluripotent stem cells via temporal modulation of canonical Wnt signaling. Proc. Natl. Acad. Sci. U. S. A. 2012; 109:E1848–1857. [PubMed: 22645348]
52. Lian X, et al. Directed cardiomyocyte differentiation from human pluripotent stem cells by modulating Wnt/β-catenin signaling under fully defined conditions. Nat. Protoc. 2013; 8:162–175. [PubMed: 23257984]
53. Malliaras K, Marbán E. Cardiac cell therapy: where we've been, where we are, and where we should be headed. Br. Med. Bull. 2011; 98:161–185. [PubMed: 21652595]
54. Lieu DK, et al. Mechanism-based facilitated maturation of human pluripotent stem cell-derived cardiomyocytes. Circ. Arrhythm. Electrophysiol. 2013; 6:191–201. [PubMed: 23392582]
55. Laflamme MA, et al. Formation of human myocardium in the rat heart from human embryonic stem cells. Am. J. Pathol. 2005; 167:663–671. [PubMed: 16127147]
56. Zhu W-Z, Santana LF, Laflamme MA. Local control of excitation-contraction coupling in human embryonic stem cell-derived cardiomyocytes. PloS One. 2009; 4:e5407. [PubMed: 19404384]
57. Ardehali R, et al. Prospective isolation of human embryonic stem cell-derived cardiovascular progenitors that integrate into human fetal heart tissue. Proc. Natl. Acad. Sci. U. S. A. 2013; 110:3405–3410. [PubMed: 23391730]
58. Shiba Y, et al. Human ES-cell-derived cardiomyocytes electrically couple and suppress arrhythmias in injured hearts. Nature. 2012; 489:322–325. [PubMed: 22864415]
59. Yang X, Pabon L, Murry CE. Engineering adolescence: maturation of human pluripotent stem cell-derived cardiomyocytes. Circ. Res. 2014; 114:511–523. [PubMed: 24481842]
60. Zahanich I, et al. Rhythmic beating of stem cell-derived cardiac cells requires dynamic coupling of electrophysiology and Ca cycling. J. Mol. Cell. Cardiol. 2011; 50:66–76. [PubMed: 20920509]
61. Satin J, et al. Calcium handling in human embryonic stem cell-derived cardiomyocytes. Stem Cells Dayt. Ohio. 2008; 26:1961–1972.

62. Kehat I, et al. Electromechanical integration of cardiomyocytes derived from human embryonic stem cells. Nat. Biotechnol. 2004; 22:1282–1289. [PubMed: 15448703]
63. Matsushita T, et al. Formation of cell junctions between grafted and host cardiomyocytes at the border zone of rat myocardial infarction. Circulation. 1999; 100:II262–268. [PubMed: 10567314]
64. Reinecke H, Zhang M, Bartosek T, Murry CE. Survival, integration, and differentiation of cardiomyocyte grafts: a study in normal and injured rat hearts. Circulation. 1999; 100:193–202. [PubMed: 10402450]
65. Zheng S-X, et al. Comparison of cardiac stem cells and mesenchymal stem cells transplantation on the cardiac electrophysiology in rats with myocardial infarction. Stem Cell Rev. 2013; 9:339–349. [PubMed: 22544360]
66. Hwang HJ, et al. Antiarrhythmic potential of mesenchymal stem cell is modulated by hypoxic environment. J. Am. Coll. Cardiol. 2012; 60:1698–1706. [PubMed: 22999735]
67. Nygren JM, et al. Bone marrow-derived hematopoietic cells generate cardiomyocytes at a low frequency through cell fusion, but not transdifferentiation. Nat. Med. 2004; 10:494–501. [PubMed: 15107841]
68. Liao S-Y, et al. Proarrhythmic risk of embryonic stem cell-derived cardiomyocyte transplantation in infarcted myocardium. Heart Rhythm Off. J. Heart Rhythm Soc. 2010; 7:1852–1859.
69. Robey TE, Saiget MK, Reinecke H, Murry CE. Systems approaches to preventing transplanted cell death in cardiac repair. J. Mol. Cell. Cardiol. 2008; 45:567–581. [PubMed: 18466917]
70. Fernandes S, et al. Human embryonic stem cell-derived cardiomyocytes engraft but do not alter cardiac remodeling after chronic infarction in rats. J. Mol. Cell. Cardiol. 2010; 49:941–949. [PubMed: 20854826]
71. Shiba Y, et al. Electrical Integration of Human Embryonic Stem Cell-Derived Cardiomyocytes in a Guinea Pig Chronic Infarct Model. J. Cardiovasc. Pharmacol. Ther. 2014; 19:368–381. [PubMed: 24516260]
72. Skelton RJP, et al. SIRPA, VCAM1 and CD34 identify discrete lineages during early human cardiovascular development. Stem Cell Res. 2014; 13:172–179. [PubMed: 24968096]
73. Kattman SJ, et al. Stage-specific optimization of activin/nodal and BMP signaling promotes cardiac differentiation of mouse and human pluripotent stem cell lines. Cell Stem Cell. 2011; 8:228–240. [PubMed: 21295278]
74. Menasché P. Stem Cell Therapy for Heart Failure Are Arrhythmias a Real Safety Concern? Circulation. 2009; 119:2735–2740. [PubMed: 19470902]
75. Fukushima S, et al. Direct intramyocardial but not intracoronary injection of bone marrow cells induces ventricular arrhythmias in a rat chronic ischemic heart failure model. Circulation. 2007; 115:2254–2261. [PubMed: 17438152]
76. Pak H-N, et al. Mesenchymal stem cell injection induces cardiac nerve sprouting and increased tenascin expression in a Swine model of myocardial infarction. J. Cardiovasc. Electrophysiol. 2003; 14:841–848. [PubMed: 12890047]
77. Makkar RR, Lill M, Chen P-S. Stem cell therapy for myocardial repair: is it arrhythmogenic? J. Am. Coll. Cardiol. 2003; 42:2070–2072. [PubMed: 14680728]
78. Gnecchi M, Zhang Z, Ni A, Dzau VJ. Paracrine mechanisms in adult stem cell signaling and therapy. Circ. Res. 2008; 103:1204–1219. [PubMed: 19028920]

Key Points

- Candidates for cardiac cell therapy include autologous sources such as bone marrow progenitor cells, skeletal myoblasts and resident cardiac stem cells. Human pluripotent stem cells including embryonic stem cells and induced pluripotent stem cells are additional candidates with vast differentiation potential, although no clinical trial has yet tested their efficacy.
- Cell coupling and engraftment are vital to improved myocardial function.
- Mechanisms for arrhythmia in stem cell transplantation include re-entrant rhythms, automaticity that is at least in part dependent on host heart rate, non-cardiac graft contaminates and non-cellular features involving nerve sprouting and increased sympathetic innervation.
- Paracrine effects may serve a protective role.
- The method of stem cell transplantation also contributes to arrhythmogenicity, in that intramyocardial injection carries a much higher rate of arrhythmia due to disruption of the native architecture of the heart.

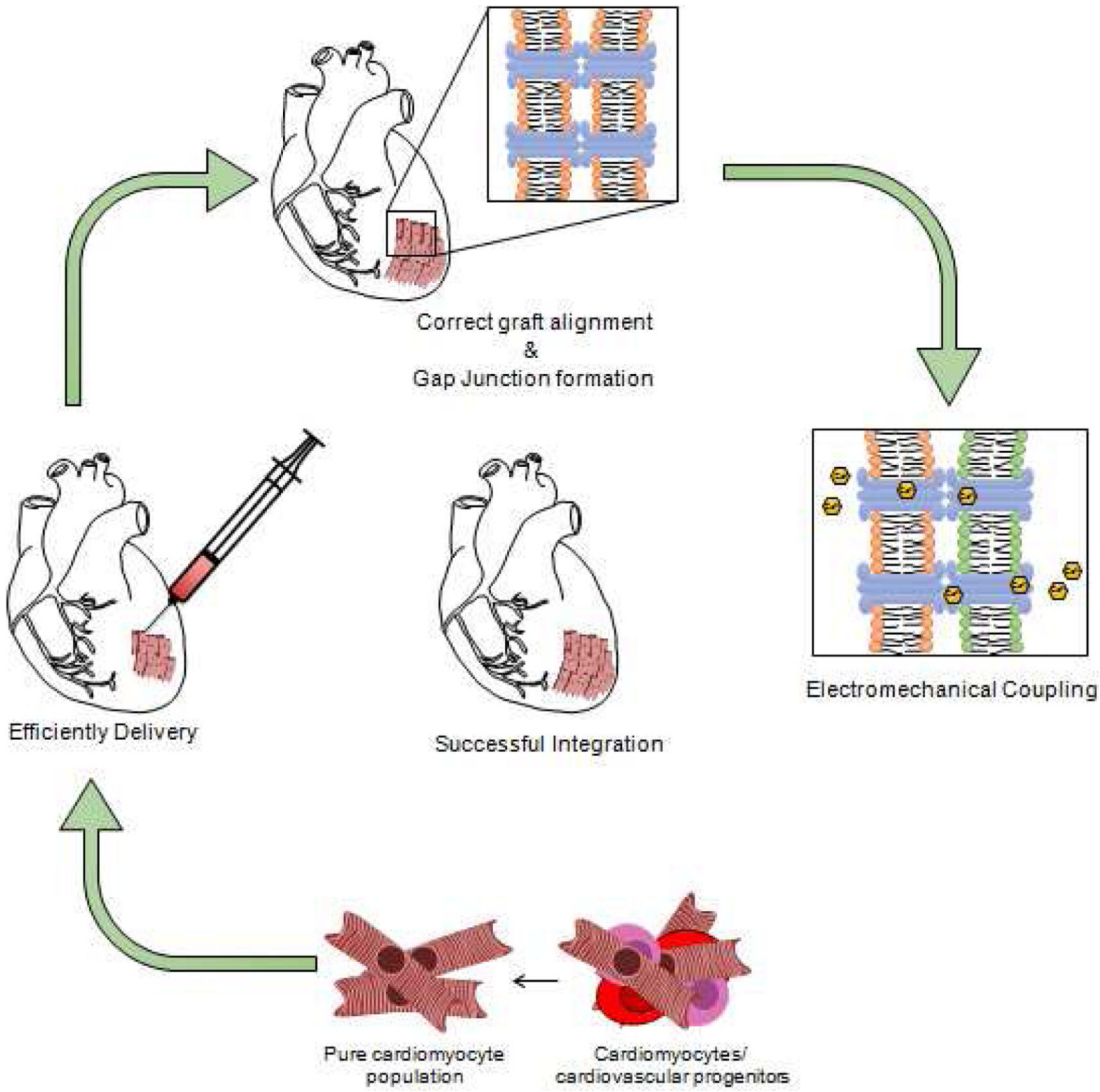

Figure 1.

Factors influencing successful graft integration. Post-transplantation, successful graft integration with host myocardium is dependent on several factors – a cell population with low percentage of non-cardiac derivatives, an efficient delivery method that favors cell survival and retention, correct graft alignment and gap junction formation that allows for electromechanical coupling.

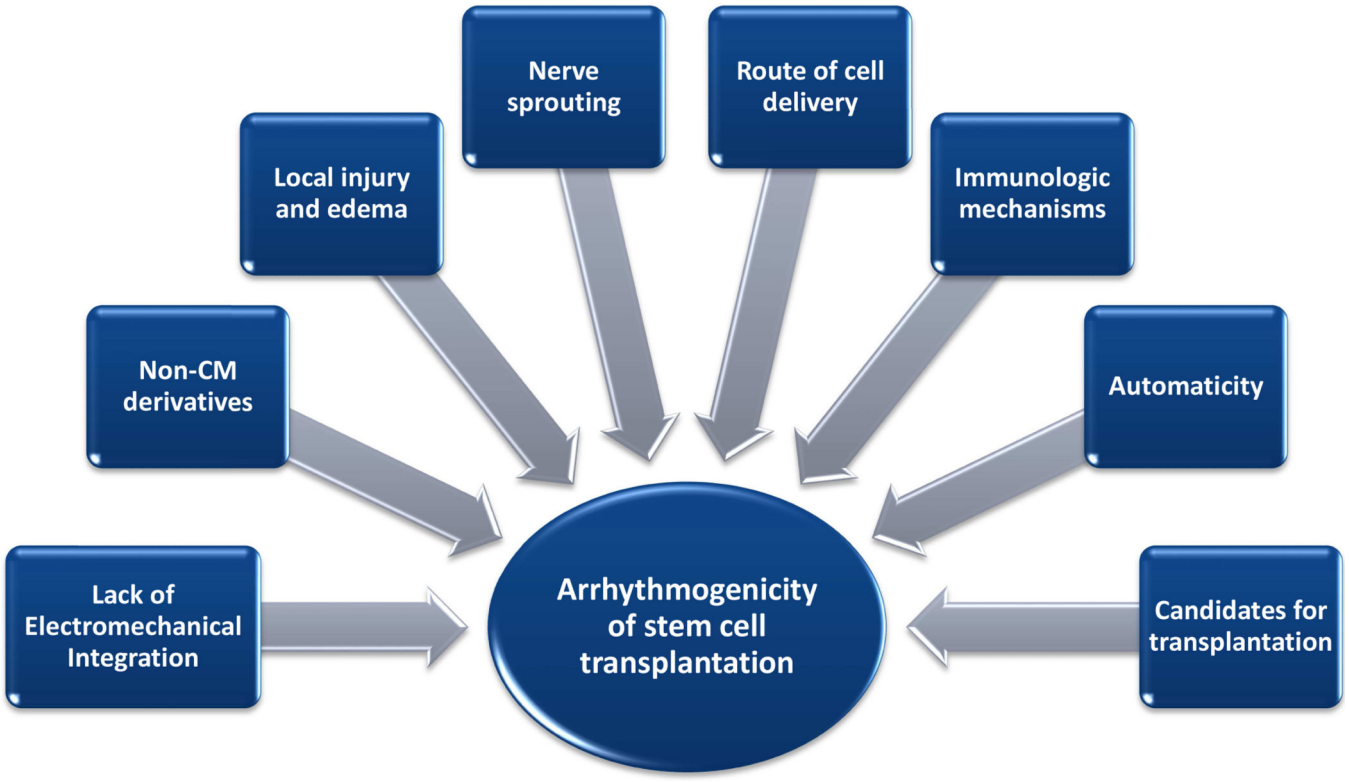

Figure 2.

Mechanisms of arrhythmogenicity. Proposed mechanisms for the higher rates of arrhythmia observed with stem cell transplantation include 1) lack of electromechanical integration; 2) transplantation of non-cardiomyocyte (CM) derivatives; 3) local injury and edema; 4) nerve sprouting resulting in increased sympathetic tone; 5) route of cell delivery, with intramyocardial being more arrhythmogenic than retrograde intracoronary; 6) immunologic mechanisms leading to rejection and inflammation; 7) graft automaticity; and 8) candidates for transplantation, where expression of gap junctions such as Connexin-43 influence the arrhythmogenicity of the graft.

Table 1

Selected active clinical trials in cardiac cell therapy

Title	Cell Type	Method	Enrollment	Objective	Primary Outcome
Allogeneic Heart Stem Cells to Achieve Myocardial Regeneration (ALLSTAR)	C-DC	Intracoronary	274	Determine whether allogeneic Cardiosphere-Derived Cells are safe and effective in decreasing infarct size in patients with MI	Infarct size assessed by MRI
Intramyocardial Transplantation of Bone Marrow Stem Cells in Addition to CABG Surgery (PERFECT)	BM-MNC	Intramyocardial	142	Determine whether intramyocardial injection of autologous CD133+ bone marrow stem cells yields a functional benefit in addition to CABG in patients with chronic ischemic CAD	Left ventricular EF at rest, measured by MRI
Effects of Intracoronary Progenitor Cell Therapy on Coronary Flow Reserve After AMI (REPAIR-ACS)	BM-MNC	Intracoronary	100	Determine whether intracoronary application of autologous bone marrow-derived progenitor cells benefits coronary flow reserve in NSTEMI	Improvement of coronary flow reserve in the infarct vessel
Autologous Bone Marrow Mononuclear Cells in the Combined Treatment of Coronary Heart Disease	BM-MNC	Intramyocardial, Intravenous	100	Evaluate the effect of the method of administration of BM-MNCs for the duration of functioning aorto-coronary bypass grafts in the surgical treatment of CAD	All-cause mortality associated with the progression of basic disease
Trial of Hematopoietic Stem Cells in Acute Myocardial Infarction (TECAM2)	BM-MNC	Intracoronary	120	Comparing intracoronary transplantation of autologous bone marrow stem-cells in ventricular post-infarction remodeling to conventional treatment or mobilization of bone marrow stem cells with G-CSF.	Change in left ventricular EF and left ventricular end-systolic volume relative to baseline measured by MRI
The Effect of Intracoronary Reinfusion of Bone Marrow-derived Mononuclear Cells on All Cause Mortality in Acute MI (BAMI)	BM-MNC	Intracoronary	3000	Demonstrate that a single intracoronary infusion of autologous bone marrow-derived mononuclear cells is safe and reduces all-cause mortality in patients with reduced left ventricular EF (</=45%) after successful reperfusion for AMI	Time from randomization to all-cause death
Compare the Effects of Single Versus Repeated IC Application of Autologous BM-MNC on Mortality in Patients With Chronic Post-infarction Heart Failure (REPEAT)	BM-MNC	Intracoronary	676	Compare the effects of single versus repeated application of autologous bone marrow-derived stem cells to treat chronic post-infarction heart failure	Mortality at 2 years after inclusion into the study
Intracardiac CD133+ Cells in Patients With No-option Resistant Angina	CD133+ Stem Cells	Intramyocardial	60	Evaluate the efficacy of autologous CD133+ cells in patients with resistant angina without the possibility of effective revascularization	Myocardial perfusion change assessed by perfusion scintigraphy
Intra-coronary Versus Intramyocardial Application of Enriched CD133pos Autologous Bone Marrow Derived Stem Cells (AlsterMACS)	CD133+ Stem Cells	Intracoronary	64	Compare the effect of intracoronary versus intramyocardial application of enriched CD133+ autologous bone marrow derived stem cells for improving left ventricular function in chronic ischemic cardiomyopathy	Change in left ventricular global EF measured via echocardiography
Repetitive Intramyocardial CD34+ Cell Therapy in Dilated Cardiomyopathy (REMEDIUM)	CD34+ PSC	Intramyocardial	80	Determine if repetitive administration of cell therapy would allow for long-lasting improvements in heart function	Change in left ventricular EF measured at baseline and 1-year

Title	Cell Type	Method	Enrollment	Objective	Primary Outcome
The Enhanced Angiogenic Cell Therapy - Acute Myocardial Infarction Trial (ENACT-AMI)	EPC	Intracoronary	100	Assess the safety and efficacy of cell and gene therapy for patients with moderate to large anterior STEMI post re-vascularization with stent implantation to the infarct related artery	Change in global left ventricular EF by cardiac MRI
Intracoronary Autologous MSC Implantation in Patients With Ischemic Dilated Cardiomyopathy	MSC	Intracoronary	80	Test the differentiation potential and therapeutic capacity of mesenchymal stem cells in severe CAD patients after intracoronary implantation	Serial monitoring of change in LVEF as measured by echocardiogram and MRI
PercutaneOus StEm Cell Injection Delivery Effects On Neomyogenesis in Dilated CardioMyopathy (The POSEIDON-DCM Study)	MSC	Transendocardial	36	Compare the safety and efficacy of transendocardial injection of autologous MSCs versus allogeneic MSCs in patients with non-ischemic dilated cardiomyopathy.	Incidence of any treatment-emergent serious adverse events 1-month post catheterization
To evaluate Efficacy and Safety of Allogeneic Mesenchymal Precursor Cells (CEP-41750) for the Treatment of CHF	MSC	Transendocardial	1730	Evaluate the efficacy and safety of allogeneic mesenchymal precursor cells for the treatment of chronic heart failure	Time to first heart failure-related major adverse cardiac events
A Randomized, Open labEled, muLticenter Trial for Safety and Efficacy of Intracoronary Adult Human MSCs in AMI (RELIEF)	MSC	Intracoronary	135	Verify the long-term efficacy and safety of the first cell treatment using hearticellgram-AMI(Autologous Human Bone Marrow Derived Mesenchymal Stem Cells) in acute MI Patients	Left ventricle ejection fraction measured 13 months after the cell treatment with MRI
Safety Study of Allogeneic Mesenchymal Precursor Cell Infusion in MyoCardial Infarction (AMICI)	MSC	Intracoronary	225	Determine the safety and feasibility of the intracoronary infusion of investigational mesenchymal precursor cells in patients with de novo anterior MI due to a lesion of the LAD	Frequency of the total major adverse cardiac and cerebrovascular events

C-DC –Cardiosphere Derived cells, BM-MNC- bone marrow mononuclear cells, MSC – mesenchymal stem cells, IC-Intra coronary, MRI – magnetic resonance imaging, CAD – coronary artery disease, CHF-Congestive Heart Failure, NSTEMI – non ST-segment elevation myocardial infarction, STEMI- ST-segment elevation myocardial infarction, CABG – coronary artery bypass graft, EF – ejection fraction, AMI – acute myocardial infarction MI – myocardial infarction, G-CSF – granulocyte colony-stimulating factor, LAD – left anterior descending, EPC-Endothelial Progenitor Cells, PSC-Peripheral Stem Cells

Data from clinicaltrials.gov.