J Physiol 594.9 (2016) pp 2511–2524

The Journal of Physiology

SYMPOSIUM REVIEW

Electrical effects of stem cell transplantation for ischaemic cardiomyopathy: friend or foe?

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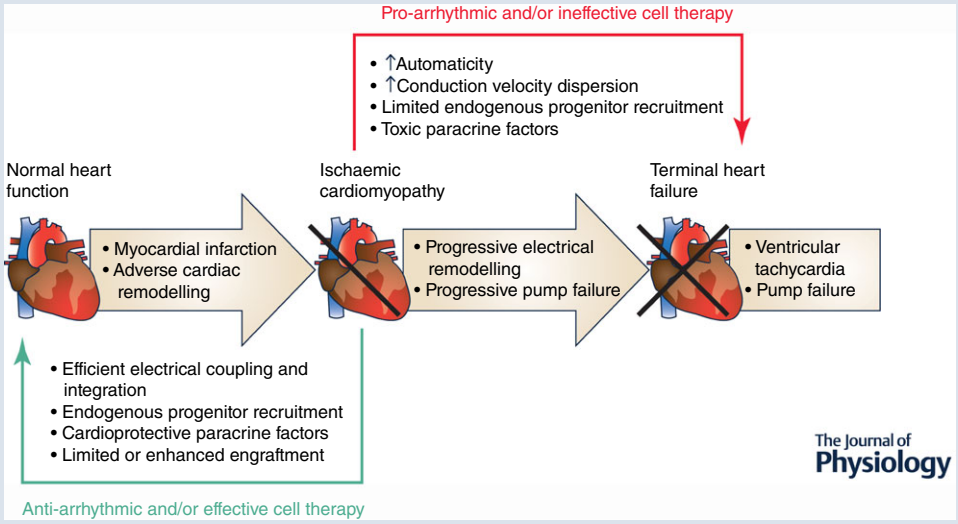

Abstract Despite advances in other realms of cardiac care, the mortality attributable to ischaemic cardiomyopathy has only marginally decreased over the last 10 years. These findings highlight the growing realization that current pharmacological and device therapies rarely reverse disease progression and rationalize a focus on novel means to reverse, repair and re-vascularize damaged hearts. As such, multiple candidate cell types have been used to regenerate damaged hearts either directly (through differentiation to form new tissue) or indirectly (via paracrine effects). Emerging literature suggests that robust engraftment of electrophysiolgically heterogeneous tissue from transplanted cells comes at the cost of a high incidence of ventricular arrhythmias. Similar electrophysiological studies of haematological stem cells raised early concerns that transplant of depolarized, inexcitable cells that also induce paracrine-mediated electrophysiological remodelling may be pro-arrhythmic. However, meta-analyses suggest that patients receiving haematological stem cells paradoxically may experience a decrease in ventricular arrhythmias, an observation potentially related to the extremely poor long-term survival of injected cells.

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This review was presented at the symposium "Cardiac Arrhythmias: Challenges for Diagnosis and Treatment. A symposium in honour of George Ralph Mines (1886–1914)", which took place at McGill University, Montreal, QC, Canada, between 6–7 November 2014.

DOI: 10.1113/JP270540

Finally, early clinical and preclinical data from technologies capable of differentiating to a mature cardiomyocyte phenotype (such as cardiac-derived stem cells) suggests that these cells are not pro-arrhythmic although they too lack robust long-term engraftment. These results highlight the growing understanding that as next generation cell therapies are developed, emphasis should also be placed on understanding possible anti-arrhythmic contributions of transplanted cells while vigilance is needed to predict and treat the inadvertent effects of regenerative cell therapies on the electrophysiological stability of the ischaemic cardiomyopathic heart.

(Received 6 August 2015; accepted after revision 18 October 2015; first published online 20 November 2015)
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Abstract figure legend Potential effects of stem cell transplantation on electrical remodeling after myocardial infarction.

Abbreviations BMC, unselected bone marrow cell; CSC, cardiac-derived stem cell; Cx43, connexin43; EDC, explant-derived cell; EPC, endothelial progenitor cell; ESC, embryonic stem cell; ESC-CM, embryonic stem cell-derived cardiomyocyte; ICM, ischaemic cardiomyopathy; iPSC, induced pluripotent stem cell; iPSC-CM, induced pluripotent stem cell-derived cardiomyocyte; LAD, left anterior descending; MSC, mesenchymal stem cell; SCID, severe combined immunodeficiency; SkM, skeletal myoblast.

Ischaemic cardiomyopathy (ICM), the most common form of heart failure, reflects underlying coronary artery disease whereby one or more myocardial infarcts convert working heart tissue to scar and progressively weaken pump function. In many ways, ischaemic cardiomyopathy (ICM) mirrors an advanced malignant disease with 27% of patients dying within 1 year of a heart failure diagnosis (Alter *et al.* 2012). Despite advances in other realms of cardiac care, the mortality attributable to ICM has only marginally decreased from 27 to 25% amongst all patients ($P = 0.03$) over the last 10 years (Yeung *et al.* 2012). These findings highlight the growing realization that current pharmacological and device therapies rarely reverse disease progression, thus rationalizing a new focus on novel means to reverse, repair, and vascularize damaged ICM hearts. To accomplish this, cell therapy has emerged as a solution to promote cardiac repair. Ideally, transplanted stem cells should display extensive engraftment and differentiation while electromechanically coupling with surrounding host myocardium to improve cardiac function. To date, a number of cell types have been explored with varying degrees of success. Evidence regarding the effects of cell transplantation on cardiac rhythm is only now beginning to emerge and is providing fascinating insights into how cardiac and non-cardiac cell sources alter the electrophysiology of the damaged/remodelled ICM heart.

Patient with ischaemic cardiomyopathy: a susceptible host to cardiac dysrhythmias

In ICM patients, ventricular arrhythmias account for significant morbidity and mortality, with premature ventricular depolarizations occurring in 70–95% of ICM patients and 50–60% of deaths attributed to an arrhythmic cause (Brodsky *et al.* 1986; Kannel *et al.* 1988). In the failing heart areas of myocardial fibrosis intermix with living tissue, creating functional and structural inhomogeneities that favour arrhythmogenesis. In most cases, the underlying mechanism is either re-entry or triggered activity.

In the case of re-entry, fibrous tissue impairs the uniform propagation of electrical impulses within the ventricle by forming complex channels in dense electrical scars. Electrical remodelling in response to abnormal intraventricular stress and strain distribution results in decreased conduction velocity through downregulation of connexin43 (Cx43) (Kitamura *et al.* 2002). As such, re-entrant circuits account for the majority of ventricular tachyarrhythmias in ICM patients.

Tissue inhomogeneities and remodelling also increase the risk of triggered activity. Abnormal depolarization may result from altered calcium handling due to increased sodium–calcium exchanger activity, leading to premature and extrasystolic beats. These effects are further compounded as greater mechanical stress is placed on non-ischaemic tissue, altering calcium handling and creating areas predisposed to increased automaticity.

As new candidate cell therapies are explored, understanding their pro- and anti-arrhythmic contributions is critical to developing safe and effective therapies for heart failure that reduce arrhythmic risk rather than create or exacerbate existing electrical instabilities.

Clinical cell candidates for cardiac repair

Over the past two decades, a number of cell products have undergone clinical consideration based on their presumed capacity to directly replace injured tissue (Table 1). Among these are skeletal myoblasts (SkMs),

Table 1. Pre-clinical effects of cell therapy on cardiac function and rhythm

Cell type	Animal model	Cell timing + delivery method	Cell transplant results	Reference
SkMs	Rats + LAD coronary artery ligation	7 days post-surgery + IC injection	13/20 SkM-treated rats had sustained ventricular tachycardia at electrophysiological testing.	Fernandes *et al.* (2006)
SkMs	Mice + cryolesion	At surgery + intra-cardiac injection	15/16 SkM-treated mice had sustained ventricular tachycardia at electrophysiological testing. Over-expression of Cx43 decreased SkM induced ventricular tachycardia by 62.5% at electrophysiological testing.	Roell *et al.* (2007)
MSCs	Pigs + catheter infarction	1 month post-infarct + catheter injection	Increased cardiac nerve sprouting no evidence for pro-arrhythmia but not formally assessed.	Pak *et al.* (2003)
BMCs	Rats + LAD coronary artery ligation	7 days post-surgery + intra-cardiac injection	No increase in sustained ventricular tachycardia at electrophysiological testing.	Fernandes *et al.* (2006)
MSCs	Pigs + catheter infarction	30 min post infarct + intravenous infusion	Improved cardiac function but decreased refractoriness 3 months post-cell transplant.	Price *et al.* (2006)
Human ESC-CMs	Nude rats + LAD coronary artery ligation	4 day post-surgery + intra-cardiac injection	Improved cardiac function with no evidence for pro-arrhythmia but not formally assessed.	Laflamme *et al.* (2007)
ESC-CMs	Mice + LAD coronary artery ligation	At surgery+ intra-cardiac injection	Improved cardiac function with no evidence for pro-arrhythmia but not formally assessed.	Ebert *et al.* (2007)
ESC-CMs	Mice + cryolesion	At surgery + intra-cardiac injection	Decrease sustained ventricular tachycardia at electrophysiological testing.	Roell *et al.* (2007)
Human ESC-CMs	Immuno-suppressed guinea-pigs + cryolesion	10 days post-surgery + intra-cardiac injection	Improved cardiac function while reducing spontaneous and inducible ventricular tachycardia	Shiba *et al.* (2012)
Human ESC-CMs	Immuno-suppressed macaques + catheter infarction	14 days post infarct + catheter injection	4/4 ESC-CM treated macaques demonstrated premature ventricular contractions and spontaneous ventricular tachycardia.	Chong *et al.* (2014)
iPSCs	Mice + LAD coronary artery ligation	At surgery + intra-cardiac injection	Improved cardiac function with no evidence for pro-arrhythmia but not formally assessed.	Singla *et al.* (2011)
Human iPSC-CM	Nude rats + LAD coronary artery ligation	At surgery	Trend to improved cardiac function with no evidence for pro-arrhythmia but not formally assessed.	Carpenter *et al.* (2012)

(*Continued*)

Table 1. Continued

Cell type	Animal model	Cell timing + delivery method	Cell transplant results	Reference
Human iPSC-CM	Immuno-suppressed pigs + LAD constriction	4 weeks post-surgery	Improved cardiac function with no evidence for pro-arrhythmia on 24 telemetry prior to sacrifice 8 weeks after cell delivery.	Kawamura *et al.* (2012)
iPSCs	Pigs + catheter infarction	7 days post-infarct	Improved cardiac function and perfusion with no evidence for pro-arrhythmia but not formally assessed.	Li *et al.* (2013)
EDCs + CDCs	Rats + LAD coronary artery ligation	At surgery + intra-cardiac injection	Both EDCs and CDCs improved cardiac function to a similar degree with no evidence for pro-arrhythmia but not formally assessed.	Davis *et al.* (2010*a*)
Human EDCs	SCID Mice + LAD coronary artery ligation	7 days post-infarct + intra-cardiac injection	Improved cardiac function with enhanced EDC engraftment and no evidence for pro-arrhythmia but not formally assessed.	Mayfield *et al.* (2014*b*)
Human EDCs	SCID Mice + LAD coronary artery ligation	7 days post-infarct + intra-cardiac injection	Diabetes impairs the EDC-mediated improvements in cardiac function with no evidence for pro-arrhythmia but not formally assessed.	Molgat *et al.* (2014)
Human EDCs + EPCs	SCID Mice + LAD coronary artery ligation	7 days post-infarct + intra-cardiac injection	Both EDCs and EPCs improved cardiac function to a similar degree with no evidence for pro-arrhythmia but not formally assessed.	Latham *et al.* (2013)
Human c-Kit$^+$ cells	SCID Mice and rats + LAD coronary artery ligation	At surgery + intra-cardiac injection	Improved cardiac function with no evidence for pro-arrhythmia but not formally assessed.	Bearzi *et al.* (2007)
Cardiospheres	Mice + LAD coronary artery ligation	At surgery + intra-cardiac injection	Improved cardiac function with no evidence for pro-arrhythmia but not formally assessed.	Messina *et al.* (2004)
Human CDCs	SCID Mice + LAD ligation	At surgery + intra-cardiac injection	CDCs improved cardiac function with no evidence for pro-arrhythmia but not formally assessed.	Smith *et al.* (2007)
Human cardiospheres + CDCs	SCID Mice + LAD ligation	At surgery + intra-cardiac injection	Cardiospheres improved cardiac function more than CDCs. No evidence for pro-arrhythmia but not formally assessed.	Li *et al.* (2010)
CDCs	Pig + catheter infarction	4 weeks post-infarct + catheter injection	CDCs improved cardiac function while not increasing inducible ventricular tachycardia on electrophysiological testing.	Johnston *et al.* (2009)
CDCs or cardiospheres	Pig + catheter infarction	4–5 weeks post-infarct + catheter injection	CDCs and cardiospheres improved cardiac function to a similar extent while not resulting in deaths (sudden or otherwise) in either group.	Lee *et al.* (2011)

haematological cell products (unselected bone marrow cells (BMCs), mesenchymal stem cells (MSCs) and endothelial progenitor cells (EPCs)), pluripotent stem cells and cardiac-derived stem cells (CSCs). In addition to the effects of direct differentiation and integration with host myocardium, there is strong evidence that transplanted cells often act indirectly, exhibiting modulatory effects on the ischaemic milieu. Already, a number of clinical trials using autologous adult progenitor cells have been completed, yielding positive and promising results (Chen *et al.* 2004; Schachinger *et al.* 2004, 2009; Makkar *et al.* 2012).

Skeletal myoblasts

SkMs represent an abundant source of skeletal progenitors (i.e. 5% of all cells located between the basal lamina and the sarcolemma of adult skeletal muscle) that were first delivered clinically to damaged hearts in 2001 (Menasche *et al.* 2001). Bolstered by their ability to be easily harvested, natural resistance to hypoxia, and formation of contractile myotubes after transplantation, SkMs served as a promising candidate to replace damaged myocardium and demonstrated long-term persistence with functional benefits in ICM patients (Hagege *et al.* 2003; Pagani *et al.* 2003). In addition to providing hints of cardiac repair, these studies also indicated that SkM grafts remained functionally and electrically isolated from surrounding host myocardium, forming areas of slow conduction which favoured re-entry (Fernandes *et al.* 2006) and resulted in a high incidence of clinical life-threatening ventricular arrhythmias (Menasche *et al.* 2003; Smits *et al.* 2003; Povsic *et al.* 2011). This pro-arrhythmic effect was owed largely to a lack of gap-junction protein Cx43 expression, the principal cardiac connexin found in ventricular myocytes (Abraham *et al.* 2005). Down-regulation of Cx43 has been demonstrated both in experimental models and in ICM patients (Ai & Pogwizd, 2005) with studies in heterozygous Cx43 knockout mice supporting the role of decreased Cx43 expression in arrhythmogenesis (Guerrero *et al.* 1997; Lerner *et al.* 2000). Efforts to overcome the electrical isolation of SkMs have shown promise, as electrical coupling and decreased arrhythmogenicity were achieved in co-cultures (Abraham *et al.* 2005) and *in vivo* (Fernandes *et al.* 2006; Roell *et al.* 2007) by inducing Cx43 expression. However, equivocal clinical benefits (i.e. no enhancement in left ventricular ejection fraction 6 months after SkM injection; Menasche *et al.* 2008) combined with ongoing concerns regarding pro-arrhythmias (i.e. the abruptly terminated MARVEL study demonstrated ventricular tachycardia in the first 4 of 7 patients receiving high dose SkMs; Povsic *et al.* 2011) combined to stall investigations regarding SkM-mediated cardiac repair. Ultimately these findings motivated the field to explore parallel investigations using autologous cell products to maximize therapeutic benefits while carefully monitoring for proarrhythmic potential or complications.

Haematological stem cell products (BMCs, MSCs or EPCs)

With the demonstration of a self-renewing population of cells in the bone marrow that possessed the ability of adopt a cardiomyogenic fate after exposure to inductive culture conditions, early interest focused on investigating the ability of haematological stem cell products to promote cardiac repair. Preclinical studies of unselected BMCs demonstrated remarkable improvements in cardiac structure and function that were initially attributed to BMC transdifferentiation into working myocytes (Jackson *et al.* 2001). Subsequent lineage tracing studies were unable to demonstrate significant transdifferentiation into cardiomyocytes despite validating equivalent benefits in cardiac function (Murry *et al.* 2004). These studies established the 'paracrine' hypothesis that pro-cardiogenic cytokines released by transplanted BMCs promoted post-infarct cardiac repair by stimulating endogenous repair and myocardial salvage (Kocher *et al.* 2001; Narmoneva *et al.* 2004; Fazel *et al.* 2006). While unselected BMCs provide modest cardiac repair in clinical trials (Jeevanantham *et al.* 2012), several strategies have been developed to improve the haematological cell products though candidate cell selection or enhanced cell culture techniques to provide EPCs or MSCs for cardiac transplantation (Davis & Stewart, 2014).

In contrast to application of SkMs to ICM hearts, the pro-arrhythmic experience with blood- and bone marrow-derived cells has been more contradictory. After transplantation, EPCs and MSCs maintain a depolarized membrane potential (−20 to −40 mV) characteristic of inexcitable cells (Heubach *et al.* 2004; Li *et al.* 2005). Unlike SkMs, they express Cx43 thus retaining the potential to electrically couple with surrounding cardiomyocytes to form inexcitable current sinks and promote electrical heterogeneity. In co-cultures with neonatal cardiomyocytes, electrically coupled MSCs remain undifferentiated and functionally isolated, thus reducing the overall conduction velocities within culture and promoting the generation of re-entrant arrhythmias (Chang *et al.* 2006). Although administration of MSCs after myocardial infarction enhances left ventricular function, intra-myocardial injection has also been shown to reduce the effective refractory period (i.e. left ventricular free wall, left ventricular peri-infarct and right ventricular free wall) and increase the spatiotemporal heterogeneity of restitution 3 months after MSC injection (Price *et al.* 2006). These findings heighten pro-arrhythmic concerns as reduced refractoriness facilitates the induction of ventricular tachycardia (Kuo *et al.* 1983; Bode *et al.* 2002) while dispersion of cardiac restitution

may contribute to electrical alternans, a harbinger of dynamic instability and the initiation of malignant ventricular arrhythmias (Weiss *et al.* 2002). In addition to introducing heterogeneity, MSCs have been shown to increase sympathetic innervation in both the atria and the ventricles 2 months after injection, potentially amplifying the effects of electrical instability and inhomogeneity (Pak *et al.* 2003). The widespread distribution of these electrophysiological and neurological changes suggests that the mechanism behind these effects is largely paracrine, linked to the early delivery of cytokines or exosomes that promote both cardiac repair and fundamental electrophysiological changes within the ICM heart.

Despite these concerning findings, haematological stem cell products have not been shown to promote arrhythmias in the 1200+ patients treated soon after myocardial infarction: rather meta-analyses suggest that patients receiving blood- or bone marrow-derived stem cells experience a decreased incidence of ventricular arrhythmias (Zhang *et al.* 2009*b*). This paradoxical observation may be linked to differential expression of cytokines/exosomes by haematological stem cells sourced from patients (as compared to non-infarcted animal models) and/or the minimal long-term engraftment seen after intra-coronary injection of haematological stem cells (i.e. long-term retention reported at $< 3\%$) (Kang *et al.* 2006; Lunde *et al.* 2006; Meyer *et al.* 2006). These findings also suggest that measures taken to improve acute engraftment may be detrimental to the observed salutary benefits derived from transplanted cells, and stem cell recipients need to be closely monitored for hints of pro-arrhythmic changes. Nonetheless, this clinical experience has motivated further investigations, including the phase III BAMI trial (Barts & The London NHS Trust, 2014), using a single infusion of bone marrow-derived mononuclear cells with the aim of reducing all-cause and arrhythmic mortality following acute myocardial infarction.

Pluripotent stem cells

The capacity to form all three germ layers and freely differentiate into all lineages has made pluripotent stem cells an appealing target for research in regenerative medicine. Embryonic stem cells (ESCs) represent cell lines derived from the inner cell mass of the blastocyst, an early pre-implantation embryo formed 4–5 days after fertilization (Thomson *et al.* 1998). ESCs can be induced to form functional cardiomyocytes *in vitro*, and electrophysiological studies reveal distinct atrial, ventricular and pacemaker phenotypes and excitation properties, even during early differentiation (He *et al.* 2003; Mummery *et al.* 2003; Satin *et al.* 2004). Even when cells are sorted for markers of ventricular lineage, significant electrophysiological heterogeneity exists including relatively depolarized resting membrane potential (-60 *vs.* -80 mV), reduced upstroke velocity (15–50 *vs.* 100–135 V s^{-1}) and shortened action potential duration at 90% of repolarization (280–400 *vs.* 250–300 ms) (He *et al.* 2003; Zhang *et al.* 2009*a*; Hartman *et al.* 2015). Small-animal studies have shown that transplantation of ESC-derived cardiomyocytes (ESC-CMs) into the infarcted heart improves contractile function and electrical conduction, reducing susceptibility to arrhythmias (Kolossov *et al.* 2006; Ebert *et al.* 2007; Laflamme *et al.* 2007; Robey *et al.* 2008; Shiba *et al.* 2012). Initial hypotheses suggested that this effect may be mediated in part through integration and electrical coupling of transplanted ESCs as exogenously delivered ESC-CMs electrically couple to the host heart in both guinea pig and primate models of myocardial infarction (Shiba *et al.* 2012; Chong *et al.* 2014). However, large-animal studies revealed that despite uniform electrically coupling between human ESC-CMs and recipient primate hearts, all animals that received ESCs experienced ventricular arrhythmias, including premature ventricular depolarizations and ventricular tachycardia (Chong *et al.* 2014). The reason for this conflicting result is unknown but it may reflect a combination of larger graft sizes and the relative immaturity of engrafted ESC-CMs. The larger ESC-derived graft sizes found in primates reflected a greater number of ESCs that could be delivered, engraft and differentiate (approximately 10-fold larger than previous small-animal studies). Given that the spread of activation within coupled immature cardiomyocytes is reduced (>50 ms for activation), this critical delay within a structurally abnormal heart may provide the ideal conditions for re-entry and pro-arrhythmia (Shiba *et al.* 2012). It is also possible that the injected cells were contaminated with ESC-derived pacemaker cells (Laflamme *et al.* 2005; Zhu *et al.* 2009) or ESC-derived cardiomyocytes with aberrant iK1 expression resulting in early and late afterdepolarizations (Lieu *et al.* 2013). Although further work is needed to establish what mechanisms account for the observed pro-arrhythmia, these findings highlight the difficulties inherent in promoting engraftment of electrically heterogeneous non-cardiac cells within an established scar.

Induced pluripotent stem cells (iPSCs) are autologous cell lines derived from cultured somatic cells after genetic reprogramming with transcription factors, microRNAs, synthetic self-replicative RNAs or small molecules (Takahashi & Yamanaka, 2006). Akin to ESCs, iPSCs have the capacity to differentiate into cells of all three germ layers, while exhibiting the same characteristic morphology and surface antigens. In addition, iPSCs have the advantage of being autologous, mitigating ethical and limiting immune rejection concerns. Akin to ESC-derived cardiomyocytes, electrophysiological profiling of iPSC-derived cardiomyocytes (iPSC-CMs)

demonstrates significant heterogeneity reminiscent of immature atrial, nodal or ventricular cells (Moretti *et al.* 2010; Zhang *et al.* 2012). The capacity for infarct repair using iPSCs has been demonstrated in numerous preclinical animal models (Carpenter *et al.* 2012; Kawamura *et al.* 2012; Li *et al.* 2013). In one such study, iPSCs demonstrated cardiac transdifferentiation equivalent with that of embryonic stem cells, along with significant inhibition of apoptosis and fibrosis and improved ventricular function (Singla *et al.* 2011). Similar functional improvements were seen using undifferentiated iPSCs injected into a porcine model (Li *et al.* 2013). Although no animals were reported lost to sudden cardiac death, the incidence of ventricular arrhythmias was not assessed. In all studies following straightforward injection of iPSCs, cell tracking has revealed modest long-term engraftment in a manner consistent with previous intra-myocardial injection studies, suggesting that the therapeutic effect of iPSCs is predominantly mediated through a paracrine release of immuno-modulatory, anti-apoptotic and proangiogenic factors (Bobis-Wozowicz *et al.* 2015).

Cardiac-derived stem cells

Almost a decade ago, the adult heart was shown to contain reservoirs of cells that express stem cell markers, propagate *in vitro* and adopt the phenotypic features of heart cells after differentiation (Oh *et al.* 2003). Since then, several studies have demonstrated that adult hearts undergo life-long replacement, with cardiomyocyte turnover estimates ranging between 0.5 and 2% per year (Soonpaa & Field, 1997; Bergmann *et al.* 2009; Walsh *et al.* 2010). While both pre-existing cardiomyocytes and endogenous stem cells have been identified as important sources for ongoing cardiomyocyte replacement, controversy exists as to which is the dominant cell source (Senyo *et al.* 2013; Torella *et al.* 2015). However, based on these findings, several groups have developed independent methods for the culture and isolation of cardiac-derived stem cells (CSCs). Among these are cardiac explant-derived cells (EDCs), antigenically selected c-Kit$^+$ cells and culture guided cardiosphere-derived cells (CDCs). The fundamental rationale for this approach is simple, with *ex vivo* amplification of resident CSCs followed by delivery to areas of injury, where they engraft and regenerate the heart.

Explant derived cells. Cardiac explant-derived cells (EDCs) represent a heterogeneous mixture of cells cultured directly from minced and plated myocardial biopsies (Davis *et al.* 2010*a*). EDCs contain complimentary subpopulations of cardiac (c-Kit$^+$), endothelial (CD31$^+$, CD34$^+$), and mesenchymal (CD90$^+$) progenitor cells that act synergistically to provide myocardial repair (Latham *et al.* 2013; Molgat *et al.* 2014; Mayfield *et al.* 2014*a*,*b*). Unfortunately, direct application of this initial cell product to the clinical setting is limited by a constant return to the proportion of explant tissue plated, thus necessitating either antigenic selection followed by *ex vivo* proliferation (i.e. c-Kit$^+$ cells) or direct culture guided expansion (i.e. CDCs) of the initial cell product.

c-Kit$^+$ cardiac stem cells. First identified in 2003, cardiac stem cells positive for the receptor tyrosine kinase c-Kit represent a small resident population within the adult mammalian heart capable of cardiomyocte, vascular smooth muscle and endothelial differentiation (Beltrami *et al.* 2003). Antigenically sorted c-Kit$^+$ stem cells have been shown to differentiate and confer functional improvements after transplantation into ischaemic myocardium in both preclinical and clinical studies, despite lacking evidence that these cells express currents typical of mature cardiomyocytes (Bearzi *et al.* 2007). In the randomized open-label SCIPIO trial, the safety and efficacy of intracoronary delivery of c-Kit$^+$ cardiac stem cells was established, revealing a 12.3% improvement in left ventricular ejection fraction in treated patients at 2 year follow-up, with no mortality or major adverse cardiac events (Bolli *et al.* 2011; Chugh *et al.* 2012). Since publication, concerns have been raised about the SCIPIO trial regarding the integrity of data demonstrating c-Kit$^+$ cell characterization (The Lancet Editors, 2014). While troubling and the subject of ongoing investigation, the trial was designed such that cultured cells were shipped to a separate hospital for administration and patient follow-up. Although this 'division of labour' has been suggested to preserve the integrity of patient outcome data, there are no immediate plans to start a Phase 2 trial involving c-Kit$^+$ cells (Grens, 2015).

Interestingly, emerging lineage tracking evidence also suggests that percentage of cardiomyocytes emerging from resident adult c-Kit$^+$ cells during development and response to cardiac injury are vanishingly small and highly unlikely to influence cardiac function (Jesty *et al.* 2012; van Berlo *et al.* 2014). While prolonged culture of c-Kit$^+$ in inductive media may influence the adoption of a cardiac phenotype, more preclinical work is needed to define the operative mechanisms and cardiogenic potential of *ex vivo* proliferated c-Kit$^+$ cells.

Cardiosphere-derived cells. Three-dimensional cardiospheres spontaneously assemble during brief exposure of EDCs to non-adherent culture conditions (Messina *et al.* 2004). Although sphering EDCs does not amplify cell numbers, these conditions promote the expression of stem cell-related antigens (c-Kit$^+$) and transcripts (Li *et al.* 2010; Davis *et al.* 2010*b*). Cardiospheres can then be expanded within adherent culture conditions

to yield cardiosphere-derived cells (CDCs). Akin to EDCs, CDCs represent a heterogeneous mixture of cells, with subpopulations expressing stromal, mesenchymal, and progenitor related surface antigens (Smith *et al.* 2007; Davis *et al.* 2009). Importantly, CDCs express Cx43, and maintain a capacity for differentiation into electrophysiologically functionally mature myocytes (Davis *et al.* 2010*b*). When cocultured with neonatal rat ventricular myocytes, human and porcine CDCs exhibit intracellular calcium transients synchronous with surrounding myocytes, spontaneous action potentials, and fast inward sodium currents, consistent with a mature ventricular phenotype (Smith *et al.* 2007). In a pre-clinical murine model of myocardial infarction, CDC therapy demonstrated superior functional benefits when compared with BMCs and MSCs (Li *et al.* 2012). In contrast to the experience with ESC-CMs, large-animal studies of transplanted cardiospheres or CDCs have failed to demonstrate significant spontaneous or inducible ventricular arrhythmias – although these effects may in part be related to modest long-term engraftment (Johnston *et al.* 2009; Lee *et al.* 2011). Finally, the phase I randomized CADUCEUS trial demonstrated safety and efficacy of CDC delivery by intracoronary infusion, showing a 12.3% reduction in infarct size in the cell-treated group after 12 months (Makkar *et al.* 2012; Malliaras *et al.* 2014).

Owing to divergent culture methods and cell products, therapeutic comparisons of CSC therapy effects are difficult. However, taken together these data provide strong evidence for the therapeutic efficacy of *ex vivo* proliferated CSCs when delivered to the ICM heart. To date, only CDCs have demonstrated electrophysiological evidence of differentiation to a mature myocyte phenotype that electrically couples with surrounding cells – albeit within inductive culture conditions and not *in vivo* (Smith *et al.* 2007).

Theoretical model of stem cell autonomous effects on ventricular arrhythmias

Emerging data from preclinical and clinical studies have provided greater insight into the effects of cardiac cell therapy on ventricular arrhythmias. Figure 1 highlights our current understanding of the interrelated mechanisms by which transplanted stem cells provide cardiac repair and how these functions contribute to either net pro- or anti-arrhythmic effects. Consider the example of SkMs (left), engrafting to directly replace damaged myocardium. As these cells lack Cx43 expression, tissue inhomogeneities arise in the form of electrically uncoupled grafts, providing a substrate for the formation of reentrant arrhythmias. Conversely, MSCs (right) through the release of paracrine factors, induce cell cycle re-entry in resident cardiomyocytes and attenuate the effects of negative remodeling conferring an anti-arrhythmic benefit- while their transient retention limits the adverse effects direct coupling to form electrical sinks or paracrine mediated electrophysiological remodeling.

Modification of stem cells to maximize therapeutic repair

The modest engraftment and functional improvements conferred by first generation autologous therapies have prompted a number of groups to explore the effects of augmenting the regenerative potential of existing cell products. To date, several studies have used direct genetic modification to enhance stem cells before transplantation (Song *et al.* 2010; Davis & Stewart, 2011). One such study, however, demonstrated reduced cell clearance via apoptosis and increased long-term retention of CSCs by overexposing Pim-1 kinase, a key modulator of Akt signalling (Muraski *et al.* 2008). Parallel efforts using genetic overexpression have been made to improve cell survival (SDF-1, Bcl-2), electrical integration (Cx43), differentiation (TGF-β), migration (CXCR4, eNOS), and vasculogenesis (HIF-1, VEGF). Attempts to translate these approaches to the clinic have been limited to date. In the phase II ENACT AMI (Enhanced Angiogenic Cell Therapy in Acute Myocardial Infarction) trial, early EPCs are transfected with human endothelial nitric oxide synthase (eNOS) prior to intracoronary delivery (Taljaard *et al.* 2010). This is the first clinical trial to employ a combination gene and cell therapy for the treatment of cardiac disease.

The most significant improvements in CSC retention and long-term engraftment have come through biomaterial approaches. Low acute retention of injected cells reflects a combination of mechanical extrusion, lymphatic and venous clearance, and off-target disbursement. Initial biomaterial approaches used matricellular materials to anchor transplanted cells within the site of injection. In addition to structural support, biosynthetic materials provide adhesion stimuli and trophic support that increase the paracrine secretion of cardioprotective cytokines and prevent cell loss due to apoptosis (Zhang *et al.* 2008). Similarly, synergistic benefits were observed when CSCs have been co-administered with synthetic platelet gels, supportive matrigel, suggestive of an enhanced paracrine profile with a superior capacity for myocardial repair (Cheng *et al.* 2012*a*,*b*). Although cells need not be embedded within a collection of excess (potentially prothrombotic material) as intra-myocardial delivery of cells encapsulated within a protective agarose supplemented cocoon have been shown to improve cell viability and proliferation, indicating that capsules supplemented with key ECM binding proteins re-establish vital cell-matrix attachments lost during mobilization for transplant (Mayfield *et al.* 2014*b*). Furthermore

embedding cells within biomaterials itself may not be necessary as significant improvements in acute cell retention and functional recovery have been demonstrated following simply applying fibrin glue at the site of injection to presumably limit mechanical extrusion (Terrovitis *et al.* 2010).

Ultimately, different cell types may have specific propensities to proceed down pro- or anti-arrhythmic pathways. For this reason, it is important to understand cell characteristics when targeting candidate therapies for improvement. For example, efforts to boost engraftment in cell types with a robust paracrine signature may be unwarranted if these cells lack the capacity for differentiation and electrical integration. Combination therapy may provide an attractive means of harnessing both direct and indirect mechanisms of repair without introducing pro-arrhythmic risk (Latham *et al.* 2013). As demonstrated by the iterative translational research progress of many cell therapy strategies (Davis & Stewart, 2014), the path from bench to bedside is rarely linear, stressing the need for vigilance to predict and treat inadvertent effects of regenerative cell therapies on the electrophysiological stability of the ICM heart.

Future directions

Since the emergence of stem cell transplantation as a means of repairing and revascularizing damaged hearts,

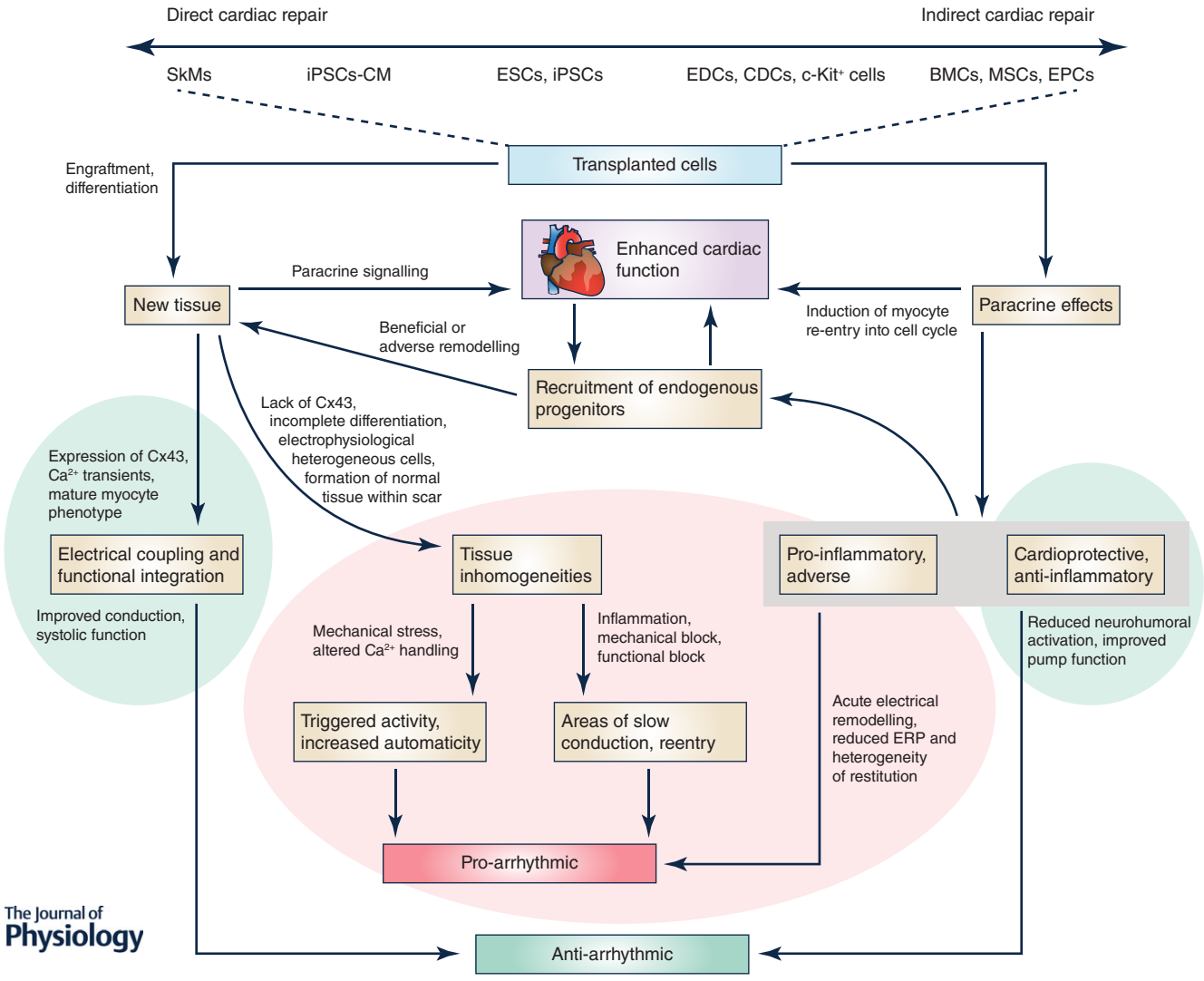

Figure 1. Schematic model of stem cell autonomous effects on ventricular arrhythmias
Candidate cell types are positioned along a continuum at the top of the figure, according to the relative extent of direct repair (engraftment + differentiation) *vs.* indirect repair (paracrine signalling) they provide. These two modes of repair are also shown (blue rectangles) below, with cardiac function serving as a mediator in between. Pro-arrhythmic contributions are highlighted in red, with anti-arrhythmic contributions in green.

several cell types have been explored in search of an ideal cellular candidate. Through a combination of direct (via differentiation) or indirect (via paracrine effects) repair, many of these technologies have yielded promising results in clinical and preclinical studies. Yet despite the existence of cells capable of forming mature cardiomyocytes, many of these cells lack the persistence and long-term engraftment required for differentiation. As efforts are made to boost cell persistence and engraftment, increasing consideration should be given to the structural and electrophysiological properties of transplanted cells and the potential for pro-arrhythmic complications. In the case of less cardiotrophic therapies, engraftment may be unnecessary or even detrimental, outweighing the cardioprotective effects of cell transplantation. As next generation therapies are developed, emphasis should also be placed on understanding possible anti-arrhythmic contributions of transplanted cells, as this may offer future therapeutic targets. The understanding of pro- and anti-arrhythmic contributions, particularly in the dynamic and unstable setting of ICM, is critical to developing safe and effective regenerative therapies.

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Additional information

Competing interests

The authors have no competing interests.

Author contributions

Both S.M. and D.R.D. drafted the article and revised it critically. All authors have approved the final version of the manuscript and agree to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

Funding

This work was supported by the Canadian Stem Cell Network and the Canadian Heart and Stroke Foundation (NA-7346). D.R.D. is funded by the Canadian Institutes of Health Research (Clinician Scientist Award MC2-121291).