Is stem cell therapy proarrhythmic?

Stem Cells Are Not Proarrhythmic
Letting the Genie out of the Bottle

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Ischemic heart disease remains a leading cause of mortality worldwide. Myocardial infarction (MI) accounts for more than half of cardiovascular-related deaths. With rising numbers of surviving MI patients and an aging population, congestive heart failure has become a major health concern, with symptomatic patients having a poorer prognosis. Despite substantial advances in medical and device strategies for congestive heart failure, there remains a growing unmet clinical need. Accordingly, various novel therapies have been explored in recent years, with the potential offered by stem cells (SCs) arguably garnering the most attention. Cardiac SC therapy has emerged as a promising option to repair ischemic/dysfunctional myocardium through a variety of potential mechanisms (Figure 1). Continuing debates over unresolved mechanistic issues and the rapid transition toward clinical application, with already-demonstrated modest but tangible benefits for cardiac structure and function, underscore the fact that the field is still in its infancy.

As with other emerging therapies, safety concerns have arisen in relation to potential hazards of SC therapy. Among the most contentious issues, the risk of proarrhythmia has sparked debate and generated lingering doubts, which run the risk of paralyzing a potentially revolutionary opportunity. Although findings that have raised concerns about proarrhythmic potential of SC transplants cannot be discounted, we contend that the available data are encouraging rather than damning. Here, we highlight experimental and clinical data on SC-related arrhythmogenesis, discuss possible confounding issues, and argue that SC therapy, rather than being considered intrinsically proarrhythmic, must be understood to have controllable proarrhythmic risks along with unique antiarrhythmic potential.

Are SCs Intrinsically Proarrhythmic?
Initial clinical trials involved satellite cells or skeletal myoblasts (SkMs) and suggested proarrhythmic potential, despite a lack of proarrhythmia in early preclinical studies. In the first phase I clinical trial using autologous SkMs, Menasché et al selected patients eligible for coronary artery bypass grafting (CABG), with severe left ventricular dysfunction (left ventricular ejection fraction [LVEF] \(< 35\%)\) and akinetic scars, to undergo multiple transepicardial cell injections to deliver an average of $87 \times 10^6$ cells. Clinically significant ventricular arrhythmias were later documented in 4 of the 9 patients included in this study (3 of whom had a history of significant prior ventricular arrhythmia), which led to the mandatory use of automatic implanted cardioverter-defibrillators. Subsequently, other trials investigating the adjunctive use of autologous SkMs in patients with chronic ischemic cardiomyopathy undergoing surgical revascularization also reported postoperative ventricular tachyarrhythmias (VTs), despite notable differences in the number of cells delivered at the time of surgery (from $0.2 \times 10^7$ to $30 \times 10^6$). With a percutaneous intramyocardial route, Smits et al reported proarrhythmic effects of autologous SkMs in postinfarction patients with depressed LVEF. Similarly, the use of bone marrow–derived cells (BMCs) has been linked, albeit to a lesser degree, with increased occurrence of VT.

The opinions expressed in this article are not necessarily those of the editors or of the American Heart Association.

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randomized 20 elective CABG patients to receive either heparinized saline or multiple intramyocardial injections of CD34+ BMCs in the border zone of a nonviable scar (as assessed by magnetic resonance imaging and thallium scintigraphy). Follow-up programmed ventricular stimulation studies were performed 6 to 8 weeks postinjection. Six of 9 BMC patients were found to have inducible VT (5 monomorphic, 1 polymorphic) compared with none of 5 controls. These preliminary reports pointed to an alarmingly higher—than-expected incidence of VTs associated with SC therapy versus contemporary post-MI subjects.25

The first wave of clinical studies primarily focused on contractile benefits of adult SCs and their potential for cardiac regeneration. Parallel experimental data began to shed light on the electrophysiological impact of SC cardiomyocyte interactions.

Although cell engraftment occurs, nested SkMs remain electrically isolated from surrounding myocardium.26,27 The failure of electromechanical coupling is attributable to insufficient gap junction formation with host tissue.28 The absence of expression by SkMs of connexin-43, the predominant gap-junction channel–forming protein in mammalian ventricular tissue, has been identified as the cause for poor functional integration.27,28 Undifferentiated SkMs express large amounts of this protein, as well as N-cadherin, but as SkMs differentiate into myotubes, these key gap junction proteins become markedly downregulated.30 Cocultured rat cardiomyocytes and human SkMs demonstrate arrhythmogenic spiral waves that are suppressed by engineering connexin-43–expressing SkMs for coculture.31 Human mesenchymal stem cells (MSCs) elicit reentrant arrhythmias on coculture with neonatal rat ventricular cardiomyocytes despite good coupling, presumably because of increased tissue heterogeneity induced by inexcitable human MSCs coupled to neonatal rat ventricular cardiomyocytes.32 Pak et al investigated the effect of injecting human MSCs and BMCs on cardiac innervation in a swine infarction model.33 Relative to control groups, pigs injected with a combination of BMCs and MSCs had greater densities of developing nerves staining positive for tenascin, a family of extracellular matrix proteins linked to cardiovascular remodeling and nerve regeneration.34–37

The investigators speculated, on the basis of prior studies linking cardiac hyperinnervation with sudden cardiac death,27 that heterogeneous sympathetic nerve sprouting imparted arrhythmia susceptibility. Finally, Zhang et al uncovered the potential for all 3 classic proarrhythmia mechanisms (reentry, triggered activity, and automaticity) in whole-cell patch-clamp studies of ESC-derived cardiomyocytes.38

**Potential Mechanisms of SC Effects on Myocardial Electrical Substrates**

The above data suggest a propensity of SC therapy to create or exacerbate VT. SC-related proarrhythmia may act alone or in concert with the arrhythmic substrate of the injured myocardium via (1) intrinsic electrophysiological properties of SCs, (2) modulated graft-host or graft-graft electromechanical coupling (or both), (3) changes in ion channel function, (4) induced heterogeneity, and (5) altered myocardial tissue architecture (Table 1). Considerations on the proarrhythmic actions of stem cells are summarized in Figure 2. However, as discussed below, the electrically active properties of SCs are potentially a double-edged sword that can also offer tools to suppress or treat cardiac arrhythmogenesis.

**Shades of SC Proarrhythmia**

The current perception of the proarrhythmic potential of SC therapy has been skewed by early disquieting reports from SkM studies in chronic ischemic cardiomyopathy. A summary of clinical trials data relating to the proarrhythmic potential of SC therapy is provided in Table 2. Without discounting these studies, it is crucial to consider whether the proarrhythmic risk is preventable or controllable (or both), whether the risk is transient, and, finally, whether the risk is shared by all candidate SC treatment approaches to cardiac repair.

It is very important to remember the inherent arrhythmic substrate of patients enrolled in the initial SkM trials. Diseased ischemic myocardium represents an ideal setting for ventricular arrhythmogenesis based on preexisting (anatomic and electrophysiological) heterogeneities, as well as dynamic instability manifested by electrical alternans, conspiring to
amplify any proarrhythmic risk.\textsuperscript{39} Although VT is rather uncommon among CABG patients, specific subsets of patients are particularly at risk. The combination of prior MI, severe symptomatic heart failure, and low left ventricular ejection fraction (LVEF < 40%) identifies CABG patients with a 30% likelihood of developing VT, versus 1% when none are present.\textsuperscript{40}

Although a firm causal relationship between SC therapy and VT is difficult to ascertain from the initial, nonrandomized, and noncontrolled studies, heightened awareness of proarrhythmic risk associated with SkMs led investigators to introduce prophylactic amiodarone therapy at the time of cell transplantation. Herreros et al administered oral amiodarone for 3 months to elective CABG patients (mean LVEF, 36%) injected subepicardially with SkMs into akinetic or dyskinetic tissue deemed nonviable by stress echocardiographic and positron emission tomography studies.\textsuperscript{41,42} At 1-year follow-up, beneficial effects on contractility and viability were documented without occurrence of VT. Similarly, Siminiak et al administered prophylactic amiodarone in 2 phase I (surgical and percutaneous) trials.\textsuperscript{21,43} After VT episodes were observed early after SC transplantation in the first 2 study patients, all remaining CABG patients undergoing SkM injections were given amiodarone up to 6 weeks postintervention. No subsequent arrhythmic events were documented. These data suggest that there may be a transient early

![Figure 2. Issues to consider when assessing the potential proarrhythmic effect of stem cells.](http://circ.ahajournals.org/issue/April/2009/1826/Circulation_April_7_2009.pdf)
Table 2. Skeletal Myoblast Clinical Trials and Arrhythmic Side Effects

<table>
<thead>
<tr>
<th>Study (Year)</th>
<th>Study Type</th>
<th>Patients, n (Active/Control)</th>
<th>LVEF, Mean or Entry Criteria, %</th>
<th>Concomitant Procedure</th>
<th>Intramyocardial Delivery Route</th>
<th>Autologous Serum</th>
<th>Amiodarone Prophylaxis</th>
<th>Serious VT, Yes or No (n or Active/Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Menasché (2003)¹⁷</td>
<td>Nonrandomized</td>
<td>10 (10/0)</td>
<td>15–35</td>
<td>CABG</td>
<td>Transepicardial</td>
<td>No</td>
<td>No</td>
<td>Yes (4)</td>
</tr>
<tr>
<td>Pagani (2003)²⁵</td>
<td>Nonrandomized</td>
<td>5 (5/0)</td>
<td>5–25</td>
<td>LVAD</td>
<td>Transepicardial</td>
<td>No</td>
<td>No</td>
<td>Yes (3)</td>
</tr>
<tr>
<td>Smits (2003)²³</td>
<td>Nonrandomized</td>
<td>13 (13/0)</td>
<td>20–40</td>
<td>Catheterization</td>
<td>Endomyocardial</td>
<td>No</td>
<td>Yes</td>
<td>Yes (4)</td>
</tr>
<tr>
<td>Herreros (2003)⁴¹</td>
<td>Nonrandomized</td>
<td>12 (12/0)</td>
<td>≥25</td>
<td>CABG</td>
<td>Transepicardial</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Chachques (2004)⁴⁴</td>
<td>Nonrandomized</td>
<td>20 (20/0)</td>
<td>28</td>
<td>CABG</td>
<td>Transepicardial</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Ince (2004)⁵²</td>
<td>Nonrandomized</td>
<td>12 (6/6)</td>
<td>20–40</td>
<td>Catheterization</td>
<td>Endomyocardial</td>
<td>No</td>
<td>Yes</td>
<td>Yes (2)</td>
</tr>
<tr>
<td>Siminiak (2004)⁴³</td>
<td>Nonrandomized</td>
<td>10 (10/0)</td>
<td>25–40</td>
<td>CABG</td>
<td>Endomyocardial</td>
<td>No</td>
<td>Yes</td>
<td>Yes (2)</td>
</tr>
<tr>
<td>Siminiak (2005)⁴³</td>
<td>Nonrandomized</td>
<td>10 (10/0)</td>
<td>25–40</td>
<td>Catheterization</td>
<td>Transvenous</td>
<td>No</td>
<td>Yes</td>
<td>Yes (1)</td>
</tr>
<tr>
<td>Dib (2005)²²</td>
<td>Nonrandomized</td>
<td>24 (24/0)</td>
<td>28</td>
<td>CABG-LVAD</td>
<td>Endomyocardial</td>
<td>No</td>
<td>No</td>
<td>Yes (3)</td>
</tr>
<tr>
<td>Dib (2007)*</td>
<td>Randomized</td>
<td>23 (12/11)</td>
<td>≤40</td>
<td>Catheterization</td>
<td>Endomyocardial</td>
<td>No</td>
<td>N/A</td>
<td>Yes (1/1)</td>
</tr>
<tr>
<td>Sherman (2008)†</td>
<td>Nonrandomized</td>
<td>20 (20/0)</td>
<td>20–40</td>
<td>Catheterization</td>
<td>Endomyocardial</td>
<td>No</td>
<td>No</td>
<td>Yes (4)</td>
</tr>
<tr>
<td>Menasché (2008)⁶⁵</td>
<td>Randomized</td>
<td>97 (67/30)</td>
<td>15–35</td>
<td>CABG</td>
<td>Transepicardial</td>
<td>No</td>
<td>Yes</td>
<td>Yes (9/2)</td>
</tr>
</tbody>
</table>

VT indicates ventricular tachyarrhythmias; LVAD, left ventricular assist device.

Arrhythmic risk, possibly due to inflammatory reactions or irritative effects (or both) of intramyocardial injection. An additional potential consideration is the use of autologous serum for cell expansion and delivery. In contrast to prior studies, Herreros et al avoided xenogenic serum interaction with cultured SKMs, with an apparently reduced proarrhythmic profile. Chachques et al subsequently noted no malignant VT in 20 patients after transplantation of SKMs when the latter were expanded in the patients’ own serum.¹⁴

Menasché et al then initiated the multicenter, randomized, placebo-controlled Myoblast Autologous Grafting in Ischemic Cardiomyopathy (MAGIC) trial, a dose escalation study of SKM injections in patients undergoing elective CABG (LVEF 15% to 35%). All patients underwent mandatory automatic implanted cardioverter-defibrillator implantation. Doses of 400 or 800×10⁶ cells/patient (n=33 and n=34, respectively) were compared with placebo (n=30). The study was prematurely terminated for failure to meet the primary efficacy end point (6-month echocardiographic changes in contractility). However, a statistically significant improvement in left ventricular remodeling was observed among high cell transplant number versus placebo patients. On the basis of blinded automatic implanted cardioverter-defibrillator interrogations, no death was attributed to arrhythmic events, although a non–statistically significant greater number of VTs in SKM-treated patients (n=2, placebo group; n=4, low cell group; n=5, high cell group) was observed. Of note, no dose–response risk for proarhythmia or time to first arrhythmic event was discovered.

Clinical trials using BMC transplants in the setting of recent MI (<14 days) have suggested functional benefits (eg, improved LV remodeling, reduction in infarct size). Varying results have been explained by differences in timing of administration or quality of cell harvest (or both). Although sustainability of benefits remains controversial, Most BMC studies have focused on patients with preserved LVEF, and only a handful have investigated end-stage congestive heart failure patients or those not amenable to standard revascularization strategies. Whether administered by surgical or percutaneous methods, via intracoronary or intramyocardial routes, early or remotely after acute MI, using selected or unfractionated BMCs, or as a combination of BMC subtypes, BMC therapy studies have generally shown a lack of VT events as evaluated by serial 24-hour Holter monitoring or programmed simulation studies (or both).

In parallel, insights gained from experimental data have helped to refine our understanding of the proarrhythmic risk. These studies have confirmed a greater propensity for SKMs (compared with BMCs) to generate ventricular tachyarrhythmias. Using optical mapping studies, Mills et al found that, while SKMs invariably induced arrhythmias following injection, engrafted MSCs preserved electrical viability, maintained impulse propagation along the border zone and showed decreased VT inducibility. Injection may be a key consideration: Fouts et al compared the injection of SKMs in scar tissue compared with neighboring viable regions and found increased VT-propensity after border-zone compared with central-scar injections. The route of delivery is also important. Fukushima et al found that when compared with direct intramyocardial injection, myoblast delivery via retrograde intracoronary injection produced more...
Electrical coupling can occur between cardiomyocytes and nonmyocyte cells such as cardiac fibroblasts. Exogenous SCs could conceivably couple with host cardiac tissue through a span of a few nonmyocyte cells or even act as conduction bridges, given that variable degrees of structural coupling have been reported for both SkMs and MSCs.

Far from “proarrhythmic,” SCs may have frank “prorhythmic” (antiarrhythmic) properties against both brady- and tachyarrhythmias. Engineered human MSCs overexpressing the pacemaker channel gene mHCN2 form gap junctions with surrounding host tissue and generate spontaneous rhythms. Another innovative alternative to creating biological pacemakers is to drive human ESC differentiation toward early-stage cardiomyocytes with pacemaker properties.

Space does not permit a detailed discussion of SC-approaches to abolishing tachyarrhythmias, but the interested reader is referred to recent relevant articles. The possibilities opened by specific targeting of engineered SC therapy to arrhythmic tissues offer unparalleled potential for innovative ventricular antiarrhythmic therapy. Finally, clinical trials have begun to incorporate electrophysiological considerations of SC therapy to address the close relationship between electrical activation and ventricular function. After intracoronary delivery of unfractionated BMCs, Chang et al documented an impact of SC therapy not only on exercise capacity and infarct volume but also on LV systolic synchrony, as defined by the time to peak positive systolic velocity standard deviation, a reliable parameter of LV synchronicity in ischemic cardiomyopathy. These intriguing findings may provide novel alternatives for cardiac resynchronization therapy and will certainly be the object of future investigation.

Conclusions
In recent years, SC biology has produced a paradigm shift in therapeutic options for a multitude of cardiovascular diseases. By highlighting the electrophysiological implications of SC therapy, an awareness of proarrhythmic risk has helped to move this emerging field forward. Future studies will consider the intrinsic electrophysiological properties of SCs, track and modulate both physical and electrophysiological integration with cardiac tissue, and address any proarrhythmic potential. Ultimately, these efforts will pave the way to harnessing both the healing and antiarrhythmic potential of SC. Thus, it has become clear that it is no longer a question of if but of how the genie will be let out of the bottle.

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