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EDITORIAL COMMENTARY

Cardiac stem cell therapy: Checkered past, promising future?

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See related article on pages 3180-8.

In this issue of the *Journal of Thoracic and Cardiovascular Surgery (JTCVS)*, Luo and colleagues¹ have demonstrated a novel approach to transplantation of human embryonic stem cell grafts within infarcted myocardium in an animal model. We commend their emphasis on transplanting stem cell grafts as directionally oriented, infarct-spanning bands. Often missing from basic science laboratories is the understanding of the importance of these macroscopic helical Torrent-Guasp myocardial bands² that allow for torsional deformation of the left ventricle during systole and, thus, appropriate ventricular function. By submitting their work to *JTCVS*, Luo and colleagues recognize that cardiac surgeons, who handle the human heart on a near daily basis, can best appreciate the importance of the heart's macro-architecture for effective cardiac function.

Their demonstrated effects of CoPP preconditioning represent an important step forward in our understanding of the basic mechanisms and principles underlying regenerative stem cell therapy. This bench-top research is crucial to

assist clinicians in achieving better clinical outcomes with cardiac stem cell therapy. In light of this success, however, it is essential to recognize the often-frustrating discordance between results of promising basic science research and more humbling clinical trials over the last 15 years of investigation into stem cell therapy for cardiac disease.

Extensive research efforts have investigated the potential for stem cell-mediated cardiac tissue regeneration in the treatment of cardiovascular disease.³ Early basic science reports suggested enormous therapeutic potential,⁴ but results from clinical trials have been disappointing to date. Basic investigators have employed various kinds of stem cells: embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), and adult progenitor cells (including skeletal myoblasts, bone marrow mononuclear cells [BMMNCs], and cardiac stem cells [CSCs]). So far, however, completed clinical trials have only reported transplantation of the various adult progenitor cells. In fact, a casual review of the clinical trials database (clinicaltrials.gov/ct2/home) shows that the overwhelming majority of trials has employed or will employ adult progenitor stem cells rather than truly pluripotent ESCs or iPSCs.

An early clinical trial of skeletal myoblasts (Myoblast Autologous Grafting in Ischemic Cardiomyopathy [MAGIC]⁵) was discontinued prematurely after both a lack of efficacy and an increased risk for arrhythmias in patients receiving stem cell therapy were demonstrated. A more recent skeletal myoblast trial (ie, SEISMIC⁶) did not reveal any effect of stem cell therapy on the left-ventricular ejection fraction (LVEF). Although BMMNCs have been the most commonly employed cell type for stem cell therapy trials (eg, REPAIR-AMI,⁷ TIME,⁸ POSEIDON⁹), these investigations have also yielded underwhelming results. Meta-analyses of BMMNC trials

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TABLE 1. Meta-analyses of bone marrow cell therapy for cardiac disease, encompassing 3 major indications¹⁰⁻¹²

Study	Indication	No. of trials	No. of patients	Δ LVEF (%)	Notes
Fisher et al (2013) ¹⁰	Refractory angina or HF	9	659	+3.47 ± 1.59	Reduced mortality with cell therapy (RR = 0.33, 0.17-0.65)
Kandala et al (2013) ¹¹	Chronic heart failure	10	519	+4.48 ± 2.05	Intramyocardial injection may be superior to intracoronary infusion in patients with LV systolic dysfunction
Delewi et al (2014) ¹²	Acute STEMI	16	1641	+2.55 ± 0.72	Improvement better in young patients (<55 y) and patients with low LVEF (<40% baseline)

LVEF, Left ventricular ejection fraction; HF, heart failure; RR, relative risk; LV, left ventricle; STEMI, ST-segment elevation myocardial infarction.

for various cardiac disease indications have demonstrated only minimal increases in LVEF across the board (Table 1¹⁰⁻¹²). Two trials implementing CSCs (SCIPIO¹³ and CADUCEUS¹⁴) were ineffective in improving meaningful clinical outcomes. Additional trials with adult progenitor cells are currently ongoing, including the ATHENA trial,¹⁵ which is utilizing adipose-derived regenerative cells for transplantation.¹⁶

After this surge of clinical trials employing adult progenitor stem cells yielded largely disappointing results, research efforts have been wisely redirected from the clinic back to the laboratory. In this effort to better understand the underlying principles of cardiac stem cell therapy, basic scientists and clinicians have learned from the mistakes of prior trials and sought solutions to challenging questions in the field, but many questions remain about basic mechanisms of stem cell therapy in cardiac regeneration.¹⁷ Garbern and Lee¹⁸ have recently outlined several of these questions expertly, 4 of which we highlight below:

1. What is the precise mechanism of action by which stem cell transplantation may demonstrate clinical efficacy?

Perhaps the most intuitive explanation for the therapeutic effects of stem cell transplantation is what we call the Cardiomyocyte Replacement Theory. According to this theory, transplanted stem cells differentiate into functional cardiomyocytes that integrate into the existing myocardial architecture to increase cardiac function through additional mechanical force. Nevertheless, data have consistently indicated that the vast majority of transplanted adult progenitor stem cells do not persist in their new ischemic environment for a prolonged period of time. Long-term engraftment rates have been shown to be <1%. These rates are so low because 90% of delivered cells are washed out of the heart within 24 hours, and approximately 90% of any remaining cells die within the first week of transplant.^{3,17} In fact, the beneficial effects of stem cell transplantation often paradoxically outlast the survival of transplanted stem cells in the ischemic heart tissue. Therefore, a second theory, which emphasizes unspecified paracrine effects of transplanted stem cells, has recently gained traction.¹⁹

This theory, which we call the Cardiomyocyte Repair Theory, suggests that trophic factors released by stem cells stimulate existing cardiomyocytes, leading to an increase in cardiac function.

It remains to be determined which specific factors are actually released by stem cells or how they promote beneficial cardiac effects. Proposed mechanisms of the Cardiomyocyte Repair Theory include stimulation of endogenous cardiac progenitor cell populations, enhanced tissue repair, improved angiogenesis, and matrix remodeling to reduce fibrosis.²⁰ Of note, a consensus¹⁸ has recently emerged that the mammalian heart is capable of creating a limited number of cardiac cells after birth and that some vertebrates, including neonatal mice, can regenerate the myocardium after experimentally induced ischemia.²¹ Various mechanisms have been proposed to explain how cardiomyocytes may innately regenerate following injury, as summarized in Figure 1. These include a signaling cascade that activates dormant progenitor cells, the proliferation of existing cardiomyocytes, activation of the epicardium, or the dedifferentiation of cardiomyocytes into progenitor cells that then proliferate.¹⁸ The proposed paracrine effects of stem cell therapy likely promote some or all of these innate pathways to increase function in the face of cardiac disease.

The authors of this editorial favor the Cardiomyocyte Repair Theory as compared to the Cardiomyocyte Replacement Theory to explain the effects demonstrated after transplantation of adult progenitor stem cells. In fact, with various other colleagues, 2 of the authors have previously demonstrated important findings, involving identification of both potential trophic factors and mechanisms of repair, that lend credence to the paracrine cardiac repair paradigm. With Bock-Marquette and colleagues,^{22,23} DiMaio and Thatcher identified a trophic factor, thymosin β4, that promotes cardiomyocyte migration, survival, and repair. In addition, thymosin β4 is capable of organwide activation of the embryonic coronary developmental program in the adult mammalian heart after systemic administration. With Huang, Olson, and colleagues,²⁴ DiMaio and Thatcher have also outlined how activation of the epicardium by specific transcription factors plays a role in myocardial repair following ischemic injury.

FIGURE 1. Proposed mechanisms for cardiac regeneration. Therapeutic effects of stem cells likely promote some or all of these innate pathways in the treatment of cardiac disease. (1) Paracrine factors promote proliferation of cardiomyocytes. (2) Progenitor cells activate and differentiate into cardiomyocytes. (3) Mature cardiomyocytes dedifferentiate to reenter the cell cycle and proliferate into new cardiomyocytes. (4) Activated epicardium stimulates angiogenesis and/or proliferation of cardiomyocytes. (Figure adapted from *Cell Stem Cell*, Vol. 12, Garbern JC, Lee RT, Cardiac stem cell therapy and the promise of heart regeneration, p. 692, Copyright 2013, with permission from Elsevier.¹⁸)

Therefore, we believe it is reasonable to suggest that increases in cardiac function in animal models following stem cell transplantation may be due to paracrine effects on the endogenous cardiomyocyte population rather than cardiomyocyte replacement via stem cell differentiation processes.

2. Which cell type is best suited for transplantation therapy in cardiac disease?

Although the cardiomyocyte repair theory is gaining wide acceptance as the proper explanation of the results in successful basic science investigations employing adult progenitor stem cells, the next generation of stem cell trials will likely involve transplantation of ESCs and iPSCs.²⁰ In theory, ESCs and iPSCs could be better suited for stem cell therapy because of their ability to fully differentiate into functional cardiomyocytes as compared to the more limited, less versatile cell lines used in the clinical trials previously discussed. Thus, in addition to paracrine effects, future

stem cells employed in regenerative therapy may have greater potential to persist as directionally oriented, infarct-spanning bands that will improve the mechanical performance of the left ventricle in systole.²⁰ In other words, these truly pluripotent cells could promote therapeutic effects via mechanisms uniting the Cardiomyocyte Regeneration and Cardiomyocyte Repair Theories.

Many questions remain about how truly pluripotent cells can be optimized in stem cell therapy. The ethical concerns involving ESCs may ultimately preclude their utility in the clinical setting. iPSCs circumvent this ethical controversy, but the development of a cost-effective protocol to create sufficient quantities of iPSCs for clinical therapy is currently a challenge.¹⁸ Even given a sufficient quantity of pluripotent stem cells, further investigations must determine in what state these cells should be transplanted.²⁰ Theoretically, pluripotent cells could be delivered as cardiomyocyte progenitors, fetal-like cardiomyocytes, or adult-like cardiomyocytes. Furthermore, these cells could

TABLE 2. Advantages and disadvantages of the various stem cell delivery methods²⁴

Method	Least invasive	Cell retention	Low risk*	Clinical experience	Notes
Epicardial	○	●	☉	☉	Delivery via mini-thoracotomy or with open heart procedure
Transendocardial	☉	●	☉	☉	Requires delivery of percutaneous catheter to left ventricle
Intracoronary	●	☉	☉	●	Preferred method during acute MI
Retrograde coronary sinus	☉	☉	☉	☉	Optimal in cases of severe coronary stenosis
Intravenous	●	○	●	☉	Very low retention due to cell trapping in lungs

MI, Myocardial infarction. *Common risks include perforation of the ventricular wall, cardiac arrhythmia secondary to local tissue inflammation, or embolus of the stem cells and/or viscous delivery suspension.

differentiate to become ventricular, atrial, or nodal cells, or even some combination of all 3. Much work still remains before it can be determined which option(s) may produce the greatest clinical efficacy.

3. What is the optimal delivery method for transplantation of stem cells?

Several delivery mechanisms have been previously employed including intramyocardial (epicardial or

transendocardial), intracoronary, retrograde coronary sinus, and systemic intravenous. Each method has significant advantages and disadvantages as described by Dib et al.²⁵ In exchange for increased accuracy of delivery, the most invasive delivery routes carry a risk of perforation, arrhythmia, or embolus of cells and/or the viscous delivery suspension. On the other hand, poor cell homing and retention limit the effectiveness of less invasive procedures and require the delivery of an increased number of cells to achieve a

FIGURE 2. Approaches to cardiac regeneration in heart disease. In addition to (1) stem cell delivery, several other related methods may prove to be effective therapies. (2) Tissue engineering combines cells with mechanical support and adjunctive biomaterials that promote survival. (3) Paracrine factors may be isolated from stem cell cultures and delivered as small molecules to promote repair and regeneration. (4) Noncardiomyocytes may be reprogrammed via the delivery of viruses, small molecules, or microRNAs. All of these mechanisms may promote one or more of the pathways for cardiac regeneration described in Figure 1. (Figure adapted with permission.¹⁸)

comparable therapeutic benefit. A summary of the various delivery methods is presented in Table 2. No consensus has been reached upon how best to deliver stem cells to damaged cardiac tissue,¹⁸ and it is likely that different methods may ultimately prove to be superior depending on the particular indication for stem cell therapy. For example, intracoronary delivery is the preferred route of transplant in patients suffering acute myocardial infarct.^{17,25} Again, we commend Luo and colleagues¹ for addressing lingering questions about cell delivery by employing an injectable delivery method designed to regenerate Torrent-Gausp myocardial bands. In particular, their attempt to replicate the physiologic architecture of the human heart via stem cell transplantation represents a novel solution for optimal delivery.

4. What adjuvants to cell delivery will maximize the benefits of stem cell therapy?

Recent investigations have demonstrated many methods to quantitatively assess cell engraftment and survival following delivery in the laboratory.²⁶ Experiments have consistently revealed that both engraftment and survival rates are extremely low regardless of the cell type or delivery mechanism implemented. Both cardiac blood flow, which washes away stem cells after intracoronary delivery, and myocardial contraction, which accentuates leakage of cells from an injection site, limit cell retention.²⁶ In addition, ischemic conditions severely inhibit the survival of any cells that successfully engraft by 24 hours posttransplant, as at least 90% of engrafted cells die within the first week. These factors limit total cell survival following transplant to <1% overall.^{3,17} Reasonable estimates suggest that approximately 1 billion cardiomyocytes are required to completely replace all of the cells from the working myocardium that is lost during a myocardial infarction.²⁷ At current cell survival rates, stem cell therapy would require the transfer of up to 1 trillion cells (roughly equivalent to 3% of the total number of cells in the human body) to each patient in order to completely replete the devastated cell population.

Therefore, various methods of tissue engineering have been investigated as adjuvant support to improve the persistence of stem cells following transplant. By increasing the viability of stem cells, these methods would allow for fewer stem cells to be transplanted while concurrently bettering clinical outcomes. Examples include cell co-culturing, biomaterial formulation, and genetic engineering of cells.¹⁷ Cell co-culturing involves pretreating stem cells with various cofactors that induce favorable cell-signaling pathways to increase cell survival and/or homing accuracy following transplantation. Biomaterials, in which cells are engrafted, may be applied as epicardial patches that increase cell retention and survival via mechanical scaffolding to limit cell washout or controlled release of biologic agents to support cells in ischemic environments.²⁸

Many attempts at genetic engineering of cells prior to delivery have been reported, but several concerns about this manner of adjunctive therapy have been raised.²⁶ Specifically, how can we ensure genetic manipulation increases cell survival without interfering with proper myocardial function or without introducing a significant risk of oncogenesis? Further research is needed to elucidate which adjuvant therapies can be optimally combined with stem cell transplantation to increase cardiac function.

In addition to tissue engineering of stem cells, other techniques to promote cardiac repair have been proposed. These include the direct delivery of paracrine factors isolated from stem cell colonies and the reprogramming of noncardiomyocytes into cardiomyocytes using growth factors or microRNA assays as described elsewhere.¹⁸ Figure 2 provides a summary of these approaches to cardiac regeneration therapy. Ultimately, all of these methods likely promote some combination of the innate cardiac repair pathways summarized above.

To these questions, which are currently under investigation in the laboratory, we add our own, which cannot be resolved with basic science research alone: How can clinical trials best assess stem cell therapy in a clinically significant manner? Most completed trials have published outcomes by employing metrics such as changes in LVEF, left ventricular end-diastolic volume, and/or left ventricular end-systolic volume. Many of these trials have shown statistically significant, but clinically disappointing, improvements in these metrics. We wonder, moreover, how meaningful these metrics are to clinicians and their patients, especially given the operator-dependent variability inherent in acquiring such measurements in addition to the physiologic variability of measurements of the same patient under different loading conditions. We believe that more clinically relevant outcomes, such as VO_2max and cardiac perfusion, must receive greater attention in future clinical trials. Other useful parameters include heart failure and angina classifications, quality of life metrics, and physical exercise performance.¹⁰ Of course, we cannot lose sight of the most clinically significant outcomes, morbidity and mortality, as stem cell therapy becomes more widespread.

Ultimately, the initial promise of stem cell transplantation research has not yielded as much success as initially hoped during the first round of clinical trials. More basic science investigation is required to elucidate the specific mechanism(s) by which transplanted stem cells promote cardiac regeneration and/or repair, which stem cell type is most effective, and how cells can be optimally delivered and engineered. As our understanding of stem cell therapy increases, it becomes more likely that clinical trials can produce truly meaningful results with implications for clinical practice. Another wave of stem cell clinical trials is fast approaching, as the NIH Heart, Lung, and Blood Institute teams with the Cardiothoracic Surgical Trials Network (CTSN) to fund and

perform this research in multicentered trials.²⁹ We remain cautiously optimistic that further investigations will yield more promising results than the first round of clinical trials, but we emphasize the tremendous challenges yet to be overcome before stem cell therapy can be a realistic therapeutic option for clinicians and their patients.

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