Cardiac cell therapy (CCT) holds great promise as a regenerative medicine approach for the treatment of cardiovascular diseases (CVDs). The first generation of CCTs tested various adult cell types, including skeletal myoblasts, bone marrow (BM)-derived mesenchymal stem cells (MSCs), and cardiac progenitor cells (CPCs). More recently, the advent of induced pluripotent stem cells (PSCs) led to the much-anticipated second generation of CCTs with bona fide, PSC-derived CPCs and cardiomyocytes. The bad news is that, to date, both adult and PSC-based CCTs have failed to meet their promise of directly remuscularizing and repairing the heart to a therapeutically meaningful extent. The good news is that some cell types clearly demonstrate encouraging results in terms of efficacy and safety and, more importantly, reveal a previously underestimated key role of CCT, to indirectly promote repair by regulating mechanisms of endogenous cardiac regeneration in the host.

The increasingly high burden of CVDs, coupled with the limited efficacy seen in both adult and PSC-based CCTs, and incomplete mechanistic understanding of adult human heart regeneration, have fueled disappointment, skepticism, and polarization of the field. This schism has been particularly apparent in the area of adult CCTs, which also faces a current crisis of scientific distrust. However, the interpretation that a possible stumble in research progress is proof that CCT is broken would be unscientific. As Daniel Wegner noted, “...tipping the balance toward skepticism can eradicate ideas faster than we can generate them. Eventually, we arrive at a vacuous chasm, with no theory standing and no idea left without serious chasms.”

Under this prism, it is worth exploring how the field of adult CCTs fares, compared with other regenerative medicine approaches. PSC-based CCTs offer perhaps the strongest argument against adult CCTs because of their unsurpassed ability to proliferate and differentiate into cardiomyocytes. The idea that such a trait is the premise of CCT is based on the observation that BM-MSC therapy stimulates cardiomyocyte amplification-based remuscularization mechanisms. However, experiments in more clinically relevant CVD models indicate that PSC-based direct remuscularization approaches exert effects that are more cosmetic than regenerative in nature because cardiomyocyte engraftment is not accompanied by scar resorption and regeneration. Moreover, both adult and PSC-based CCTs produce comparable improvements in cardiac function, likely indirectly, via paracrine stimulation of endogenous repair mechanisms in the host. Similarly, although gene-editing approaches offer hope for elucidating the genetic basis of CVDs, their potential application as regenerative therapy is currently limited. In addition to the technical challenges with safely and efficiently gene-editing billions of cardiomyocytes in vivo, CVDs are molecularly complex, rather than of monogenic cause. Likewise, the molecular mechanisms of cardiomyogenesis entail precise, spatiotemporal modulation of multiple signaling gradients in both cardiomyogenic and noncardiomyogenic cells, and, therefore, the possibility of developing cell-free, drug-based approaches to recapitulate such complex and dynamic processes in vivo is currently limited.

In this issue of Circulation Research, Monsanto et al. lend support to a promising strategy to address the limitations of cardiac regenerative approaches by engineering combinatorial CCTs. This idea stands on 2 pillars: (1) no single-cell population can produce all cell types that make up the human heart; and (2) both cardiomyogenic and noncardiomyogenic cells are essential for heart development and repair. Thus, engineering adult and PSC-derived cell combinations with complementary roles may more efficiently regulate endogenous regenerative pathways, compared with conventional CCT (Figure). For example, the observation that BM-MSC therapy stimulates endogenous CPCs, led to the idea of combining the 2 adult cell types for greater, synergistic effects. Indeed, this hypothesis has produced encouraging results in several large and small animal studies of CVD and is currently in a phase II, randomized, placebo-controlled trial in ischemic cardiomyopathy patients (NCT02501811). Similarly, the combination of human PSC-derived cardiomyocytes with vascular cells produces further improvements in heart repair compared with cardiomyocytes alone, likely because of enhanced stimulation of endogenous repair mechanisms. The new method by Monsanto et al. to derive 3 distinct cardiac stem cell types from within the adult human heart, could potentially foster such applications.

Using the cell-surface receptor cKit, both as a positive and negative selection marker, the authors devised a strategy to purify concurrently MSCs, CPCs, and endothelial progenitor cells from adult heart biopsies obtained during cardiac surgery. MSCs are the most abundant derivative, comprising ≈90% to 95% of the cardiac stem cell pool, and are purified via cardiomyocyte amplification-based remuscularization mechanisms. However, experiments in more clinically relevant CVD models indicate that PSC-based direct remuscularization approaches exert effects that are more cosmetic than regenerative in nature because cardiomyocyte engraftment is not accompanied by scar resorption and regeneration. Moreover, both adult and PSC-based CCTs produce comparable improvements in cardiac function, likely indirectly, via paracrine stimulation of endogenous repair mechanisms in the host. Similarly, although gene-editing approaches offer hope for elucidating the genetic basis of CVDs, their potential application as regenerative therapy is currently limited. In addition to the technical challenges with safely and efficiently gene-editing billions of cardiomyocytes in vivo, CVDs are molecularly complex, rather than of monogenic cause. Likewise, the molecular mechanisms of cardiomyogenesis entail precise, spatiotemporal modulation of multiple signaling gradients in both cardiomyogenic and noncardiomyogenic cells, and, therefore, the possibility of developing cell-free, drug-based approaches to recapitulate such complex and dynamic processes in vivo is currently limited.
as the CD105+/CD90+ fraction of cKit-negative cardiac cells. Consistent with previous reports, cardiac MSCs exhibit a fibroblastoid morphology, produce colony-forming units-fibroblast, and exhibit multilineage differentiation into adipocytes, chondrocytes, and osteocytes. However, compared with BM-MSCs, cardiac MSCs exhibit slow in vitro growth kinetics and express cardiac lineage markers, such as the zinc finger transcription factor GATA4 and smooth muscle actin. Immunologically, expression of MHC (major histocompatibility complexes) classes I and II and costimulatory molecules CD80 and CD86 are similar to BM-MSCs, but cardiac MSCs express higher levels of the costimulatory molecule CD40. It is, therefore, unclear whether cardiac MSCs are as immunoprivileged as BM-MSCs. Such differences, however, are not surprising because mouse studies indicate distinct identities for BM and cardiac MSCs, with the latter possibly representing postnatal epicardial progenitors.

The use of cKit as a CPC marker has been controversial. Recent studies identify at least 2 distinct cell types expressing cKit in the heart: a rare, cardiomyogenic cell likely of neural crest lineage, and a more abundant vasculogenic cell, possibly of mesodermal lineage. The work by Monsanto et al further supports these findings. Positive selection for cKit yields 2 stem cell types with distinct immunophenotypic and gene expression profiles. cKit+ endothelial progenitor cells are morphologically round and committed to vascular fates, as indicated by high angiogenic potential in a Matrigel-based ex vivo angiogenesis assay and expression of CD133 and PECAM1 (platelet and endothelial cell adhesion molecule 1). cKit+ CPCs exhibit spindle-like morphology and a more myogenic profile, as indicated by lack of PECAM1 and relatively higher expression of GATA4 and smooth muscle actin. However, whether CPCs retain cardiomyogenic capacity is not demonstrated. Importantly, gene expression profiling reveals striking differences between the 3 cardiac stem cell types in cytokines and extracellular matrix genes, such as SDF1, NRG1, FGF2, TIMP1, and MMP1.

The study by Monsanto et al is an important advance in cardiac regenerative medicine. First, it is a bold demonstration of cellular plasticity retained in the human heart, regardless of age, sex or health condition. Stem cells were isolated from patients ≤84 years old and experienced a range of diseases, including diabetes mellitus and coronary artery disease. Second, the ability to isolate 3 stem cell types from a single heart biopsy allows us to gain insight into the cellular composition in the adult human heart and the potential role of these unique cell types in CVD and regeneration. Because ≈70% of human heart cells are noncardiomyocytes, thorough research of their nature should be at the forefront of cardiac regenerative medicine. For example, Monsanto et al noted that some cultures failed to yield all 3 stem cell types, a finding which merits further investigation for any potential relationship to disease mechanisms. Last, the method of Monsanto et al enables the isolation and expansion of therapeutic volumes of cardiac MSCs, CPCs, and endothelial progenitor cells from a single biopsy with 80% to 90% success (≈100 million cells of each type could be manufactured in ≈10 passages). Such technology is expected to be important for engineering combinatorial CCTs, using adult and PSC-based combinations, in a manner that effectively eliminates barriers to endogenous cardiac regeneration and may eventually lead to a much-needed scientific breakthrough for the treatment of CVDs.

**Sources of Funding**

A. Vedenko is supported by a National Eye Institute (NEI) T32 Training Grant T32 EY023194.
Disclosures

K.E. Hatzistergos discloses a relationship with Vestion Inc that includes equity. Vestion did not contribute funding to this study. The other author reports no conflict.

References


Cardiac Cell Therapy 3.0: The Beginning of the End or the End of the Beginning?
Konstantinos E. Hatzistergos and Anastasia Venedko

Circ Res. 2017;121:95-97
doi: 10.1161/CIRCRESAHA.117.311293
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2017 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/121/2/95

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/