



Increased Potency of Cardiac Stem Cells Compared with Bone Marrow Mesenchymal Stem Cells in Cardiac Repair

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ABSTRACT

Whereas cardiac-derived c-kit⁺ stem cells (CSCs) and bone marrow-derived mesenchymal stem cells (MSCs) are undergoing clinical trials testing safety and efficacy as a cell-based therapy, the relative therapeutic and biologic efficacy of these two cell types is unknown. We hypothesized that human CSCs have greater ability than MSCs to engraft, differentiate, and improve cardiac function. We compared intramyocardial injection of human fetal CSCs (36,000) with two doses of adult MSCs (36,000 and 1,000,000) or control (phosphate buffered saline) in nonobese diabetic/severe combined immune deficiency mice after coronary artery ligation. The myocardial infarction-induced enlargement in left ventricular chamber dimensions was ameliorated by CSCs ($p < .05$ for diastolic and systolic volumes), as was the decline in ejection fraction (EF; $p < .05$). Whereas 1×10^6 MSCs partially ameliorated ventricular remodeling and improved EF to a similar degree as CSCs, 36,000 MSCs did not influence chamber architecture or function. All cell therapies improved myocardial contractility, but CSCs preferentially reduced scar size and reduced vascular afterload. Engraftment and trilineage differentiation was substantially greater with CSCs than with MSCs. Adult-cultured c-kit⁺ CSCs were less effective than fetal, but were still more potent than high-dose MSCs. These data demonstrate enhanced CSC engraftment, differentiation, and improved cardiac remodeling and function in ischemic heart failure. MSCs required a 30-fold greater dose than CSCs to improve cardiac function and anatomy. Together, these findings demonstrate a greater potency of CSCs than bone marrow MSCs in cardiac repair. *STEM CELLS TRANSLATIONAL MEDICINE 2012;1:116–124*

INTRODUCTION

Currently, there is major enthusiasm for the notion that cells may form the basis of a new class of therapeutics for cardiac injury [1]. Major new insights gleaned from both experimental studies and clinical trials [2] support a growing body of evidence indicating that this approach is safe, practical, and provisionally effective in filling a major gap in the therapeutic armamentarium for chronic heart disease [3, 4]. Whereas clinical trials have generally used bone marrow-derived cells, resulting in some improvement in cardiac function [5, 6], research has focused on a variety of cell sources, ranging from embryonic stem cells [7] and induced pluripotent stem [8] (iPS) cells to adult bone marrow and cardiac stem cells. However, major gaps in knowledge still exist about the best source of cells for cardiac repair.

Substantial progress has been made with two potential adult stem cell sources—those from the heart itself [9] and those from bone marrow [3]. In the heart, a variety of resident

stem cells have been described. These cells are characterized by either cell surface markers, such as c-kit⁺ (CD117⁺) [9], Sca-1 [10], and abcg2⁺ [11, 12], or transcription factors, such as WT1 [13] or Isl-1 [14]. Cardiac stem cells (CSCs) have been prepared by antigenic panning or culturing cardiac tissue to obtain “cardiospheres” [15]. Both bone marrow-derived [16] and cardiac-derived [17] stem cells express mesenchymal stromal/stem cell (MSC) markers, but Koninckx et al. showed that bone-marrow derived MSCs, but not c-kit⁺ CSCs, can differentiate into adipocytes and osteocytes, whereas heart-derived progenitor cells have greater cardiomyogenic potential than bone marrow-derived cells [18]. Current clinical trials are testing the safety of c-kit⁺ cells amplified after antigenic panning [19] or expanded in culture as a tissue mixture [20, 21]. Bone marrow-derived MSCs amplified in culture exhibit a constellation of effects contributing to tissue repair, including multilineage differentiation capacity, antifibrotic effects,

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paracrine signaling, and immunomodulatory effects [3, 4, 22–27].

We tested the prediction that CSCs would have greater efficacy than MSCs for cardiac repair following acute myocardial infarction (MI) by performing rigorous *in vivo* comparisons of the efficacy and biologic activity of these two stem cell sources. We studied primarily freshly isolated *c-kit*⁺ CSCs to avoid the loss of *c-kit* expression that normally occurs upon passage of these cells [28], so as to focus on the potential of these cells for engraftment and differentiation and to repair both cardiac structure and function. We also used adult-cultured cardiac CSCs for additional comparative studies.

MATERIALS AND METHODS

An expanded Methods section is available in the supplemental online data.

Isolation of C-Kit⁺ CSCs From Human Heart Tissue

Using magnetic-activated cell sorting (MACS), CD117-positive cells were separated from human fetal heart tissues and were suspended in phosphate-buffered saline (PBS) prior to injection. Adult CSCs were cultured from explanted human left-ventricular assist device cores using the method of magnetic microbeads coupled with specific (anti-human CD117) antibodies [28].

Mesenchymal Stem Cells

Human bone marrow aspirates were obtained from AllCells LLC (Emeryville, CA, <http://allcells.com>). Low-density mononuclear cells were separated from human bone marrow aspirates and cultured as described (see supplemental online data). Adherent MSCs were frozen at passage 4 or 5 and thawed prior to injection.

Animal Studies

All experiments on live animals were performed in accordance with the protocol approved by the Institutional Animal Care and Use Committee at the University of Miami. Briefly, myocardial infarction was produced in nonobese diabetic/severe combined immune deficiency mice by permanent ligation of the left anterior descending coronary artery [29]. CSCs (36×10^3 cells, $n = 17$) or human adult bone marrow-derived mesenchymal stem cells (MSCs), either 36×10^3 (low-dose, $n = 16$) or 1×10^6 cells (high-dose, $n = 17$), were delivered through intramyocardial injection immediately after MI. Nineteen mice received PBS as control. In addition, a group of mice received adult human CSCs (36×10^3 cells, $n = 10$). Animals were followed for 8 weeks after injection.

Echocardiography

Cardiac function and anatomy were evaluated by serial echocardiography at baseline and at 48 hours and 1, 2, 4, and 8 weeks following injection. End diastolic volume (EDV), end systolic volume (ESV), and ejection fractions were calculated and compared.

Hemodynamic Study

Eight weeks after injections, pressure volume loops were recorded using a Millar conductance manometry catheter (Millar Instruments, Inc., Houston, TX, <http://millar.com>) at baseline and during vena cava occlusion.

Immunohistochemistry

Hearts were harvested after hemodynamic recording and were fixed in formalin. Scar size was defined by measuring the perimeter of the Trichrome-Masson (TM)-stained scar. Alu staining BioGenex (San Ramon, CA, <http://www.biogenex.com>) was used to detect injected human cells in murine tissue. Costaining for cardiac proteins was performed to assess vascular or myocyte differentiation.

Quantification of Cellular Engraftment

We used two midventricular sections of heart, 1.5 and 3 mm below the left atrial edge. After Alu staining, the slides were scanned with a Zeiss Mirax150 fluorescent scanner (Carl Zeiss, Jena, Germany, <http://www.zeiss.com>). Images were analyzed with a panoramic viewer, and positive cells were counted in Photoshop CS3 (Adobe systems Inc., San Jose, CA, <http://www.adobe.com>).

RESULTS

Impact of Cell Therapy on Post-MI Remodeling

Myocardial infarction injury produced a time-dependent ventricular dilatation and dysfunction, the degree of which was similar in all groups 48 hours after injection (Fig. 1 and supplemental online Table 1). CSCs had the greatest impact on ameliorating left ventricular (LV) chamber enlargement. At 8 weeks following MI, LV EDV was $100.7 \pm 14.2 \mu\text{l}$ in the CSC group versus $128.1 \pm 15.7 \mu\text{l}$ in control ($p = .002$). EDV was also significantly better in the CSC group compared with low-dose MSC at 4 and 8 weeks ($133.5 \pm 14.5 \mu\text{l}$) ($p < .0001$, Fig. 1A). These data show that there was no significant change in the CSC-treated group after week 1 in EDV and EF. In contrast, control and low-dose MSC hearts exhibited an increase in their diastolic dimensions and a progressive decrease in ejection fraction after MI ($p < .05$). Hearts treated with high-dose MSC showed a drop in these functions between day 2 and week 2 and then remained stable ($p < .05$). ESV was also significantly reduced at 4 and 8 weeks in the CSC-injected hearts compared with control, whereas EF was augmented ($p < .05$) (Fig. 1B). In contrast, an equivalent number of MSCs was ineffective at offsetting chamber remodeling, whereas an approximately 30-fold greater dose of MSCs (1×10^6 cells) ameliorated the change in chamber size and attenuated the decline in EF similar to CSC treatment (Fig. 1).

Impact of Adult CSCs

To address the relative efficacy of adult CSCs, reported to have less potency than fetal [23, 30], we performed additional experiments using 36,000 adult CSCs ($n = 10$). The reduction in EF was ameliorated to a lesser degree than the fetal CSCs but was not different from the level achieved with the high-dose MSC group. EFs at 8 weeks were $24.05 \pm 6.9\%$ versus $22.9 \pm 2.8\%$ in adult CSCs versus high-dose MSCs, respectively.

Impact of Cell Therapy on LV Hemodynamics

We used a micromanometer-conductance catheter to measure integrated cardiovascular performance 8 weeks after MI (Fig. 2). Whereas CSCs had the greatest impact on augmenting LV contractility measured as preload recruitable stroke work (PRSW) (49.5 ± 5.7 vs. 32.5 ± 6.6 mmHg), neither dose of MSCs increased this parameter (26.3 ± 5.3 mmHg: high-dose and $20.9 \pm$

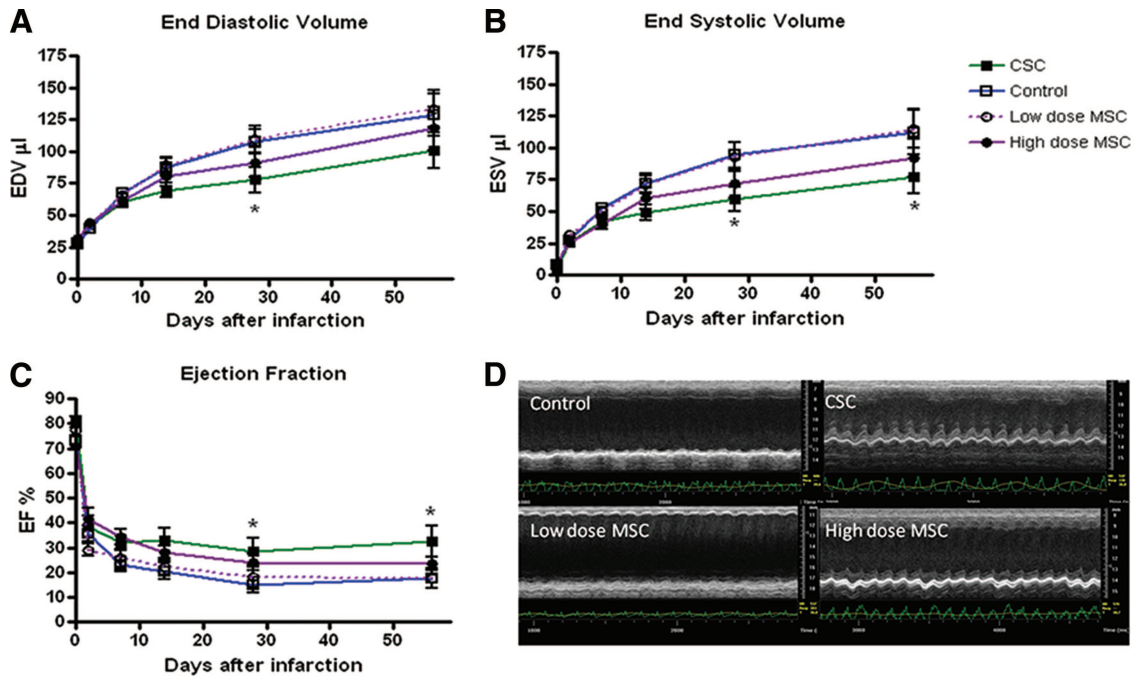


Figure 1. C-kit⁺ CSCs improve cardiovascular function as assessed by echocardiography. (A): EDV. (B): ESV. (C): EF, $p < .05$. (D): Representative echocardiographic M-mode short axis images showing preserved anatomy and function of LV in CSC-treated mice and severe dyskinesia and ventricular dilatation in control and low-dose MSC heart. *, $p < .05$. Abbreviations: CSC, cardiac stem cell; EDV, end diastolic volume; EF, ejection fraction; ESV, end systolic volume; MSC, mesenchymal stem cell.

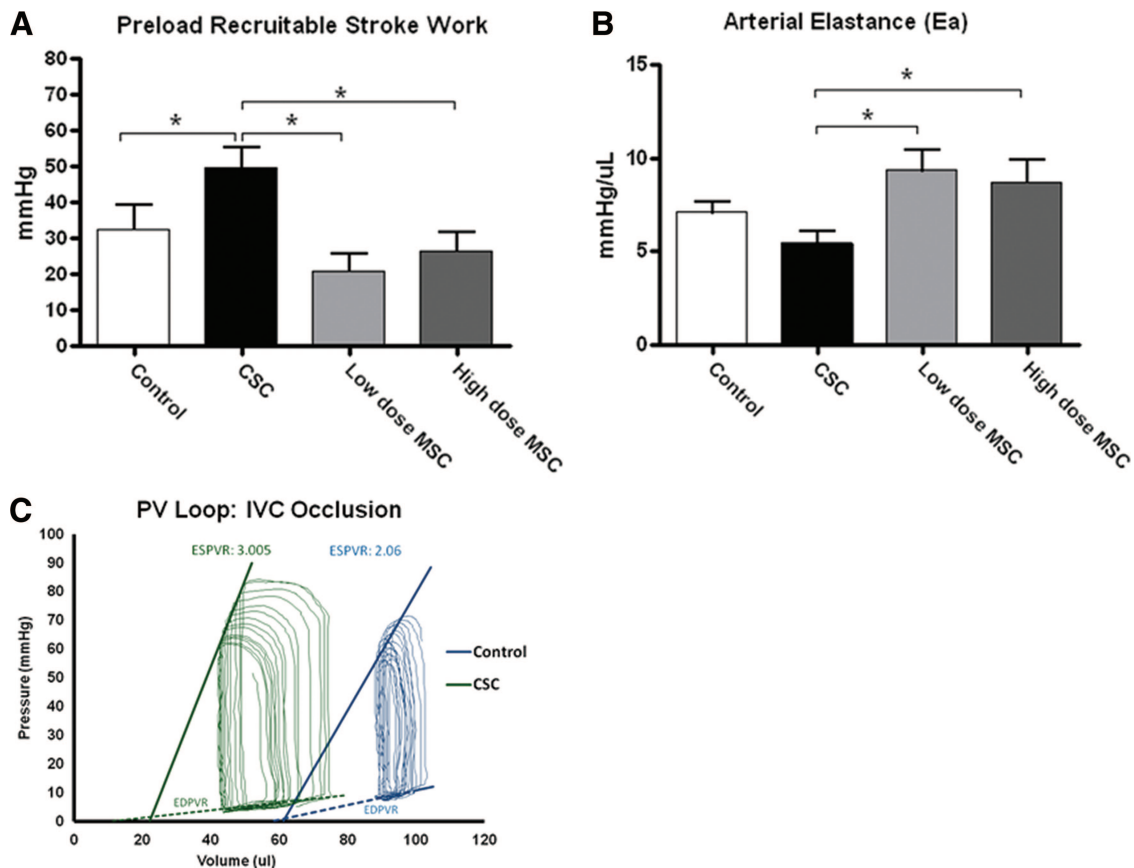


Figure 2. CSCs improve hemodynamic parameters more than MSCs. (A): Preload recruitable stroke work recorded during IVC occlusion. (B): Arterial elastance. (C): Representative PV loops showing preserved loop morphometry in the CSC-treated heart and increased ventricular volumes and decreased systolic function in control heart. *, $p < .05$. Abbreviations: CSC, cardiac stem cell; IVC, inferior vena cava; MSC, mesenchymal stem cell; PV, pressure volume.

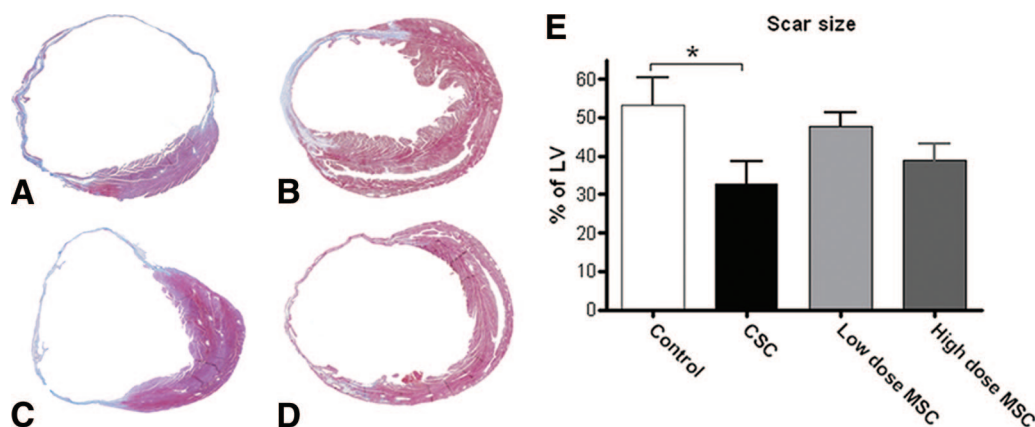


Figure 3. CSC therapy reduces scar size. Trichrome Masson (TM) staining of (A): control, (B): CSC, (C): low-dose MSC, and (D): high-dose MSC-treated hearts. (E): Quantitation of scar size following TM staining. *, $p < .05$. Abbreviations: CSC, cardiac stem cell; LV, left ventricle; MSC, mesenchymal stem cell.

4.9 mmHg; low-dose) ($p < .05$, Fig. 2A). When evaluated by the slope of the end systolic pressure volume relationship (ESPVR), contractility was increased in all cell-treated hearts: fetal CSCs (3.08 ± 0.57 mmHg/ μ l) and low-dose (3.03 ± 0.40 mmHg/ μ l) or high-dose (3.05 ± 0.49 mmHg/ μ l) MSCs versus control hearts (1.25 ± 0.36 mmHg/ μ l). Whereas CSC treatment did not influence preload (LV end-diastolic pressure was 19.6 ± 2.4 mmHg in CSCs and 22.5 ± 1.7 mmHg in controls), it did favorably affect arterial elastance (Ea), which was lower in the CSC-injected hearts (5.4 ± 0.6 mmHg/ μ l) than in either the low- or high-dose MSC-injected hearts (low: 9.3 ± 1.0 mmHg/ μ l, $p < .05$; high: 8.7 ± 1.2 mmHg/ μ l, $p < .05$), indicating that the CSC group exhibited less arterial load (Fig. 2B). Figure 2C shows a representative pressure volume loop and the corresponding ESPVR for one control and one CSC-treated animal.

Impact of Cell Therapy on MI Size

Scar size analysis using the ratio of scar to left ventricle on Trichrome Masson-stained sections showed that scar size was reduced in CSC-treated mice: $32.7 \pm 5.9\%$ versus control: $53.3 \pm 6.9\%$ ($p < .05$). Mice injected with either the low ($47.7 \pm 3.6\%$) or high ($38.7 \pm 4.5\%$) dose of MSC had scar sizes that were not significantly different from control (Fig. 3).

Cell Engraftment and Differentiation

We used human-specific DNA probes (Alu) to detect injected CSCs or MSCs in mouse cardiac tissue (Fig. 4). In stained samples, Alu⁺ cells were identified in infarcted areas (Fig. 4C), in border zones between infarcted and healthy tissue (Fig. 4B, 4D, 4F), and in remote areas away from the infarction (Fig. 4A, 4E). Alu⁺ cells were also found in proximity to blood vessel walls (Fig. 4A).

The midventricular sections of the heart 3 mm below the left atrium, a level corresponding to the location of cell injections, were used to quantify Alu⁺ cells. CSC-treated hearts (Fig. 5A) showed significantly higher incorporation of stem cells (30.3 ± 4.5 cell/mm²) compared with hearts that received the high-dose MSC treatment (0.7 ± 0.1 cell/mm², $p = .0006$) (Fig. 5B). CSCs were found primarily in three clusters that corresponded to the three injection sites. Additional clusters suggested migration of the CSCs from the injection sites (Fig. 5A). Adult CSCs exhibited lower degrees of engraftment than the fetal CSCs (data not shown).

Next, we assessed the degree of cell differentiation by co-staining with Alu sequence probes and antisera to cardiac proteins, α sarcomeric actin (α SA), and troponin I (Tnl). Using this approach, we documented that Alu⁺ cells expressed both Tnl and α SA (Figs. 4A, 4D), signifying cardiomyogenic differentiation. To confirm the localization of these cardiac markers within Alu⁺ cells, we examined laminin staining and used confocal z-stacking to identify the cell borders (Fig. 4A, 4E, 4F).

We also detected connexin 43 (Cx43) in cells that contained Alu sequence staining (Fig. 4A, 4F). The observation that these Alu⁺ cells not only express contractile proteins but also have the potential to form gap junctions suggests that the injected CSC cells underwent myocardial differentiation. In addition, groups of Alu⁺ cells that had not differentiated into cardiomyocytes were observed in the intercellular space (Fig. 4E, 4F), suggestive of reservoirs of immature cells.

In MSC-injected hearts, Alu⁺ cells were located primarily in intercellular spaces between native cardiomyocytes in the border (Fig. 6A) and remote zone (Fig. 6B). High-dose MSC hearts contained Alu⁺ cells in proximity to the vascular walls of the border zone (Fig. 6A) and the remote zone (Fig. 6B). There was no clear evidence of myocyte differentiation based upon an absence of costaining for Alu and either α SA (Fig. 6A) or Tnl (Fig. 6B). Cell engraftment was much more apparent in the high-dose MSC group compared with the low-dose group, which had only a few cells within the intercellular spaces. None of these cells costained for either Tnl or α SA, and there were no detectable cells in the vascular wall (Fig. 6C, 6D, 5B, 5C).

DISCUSSION

The major new findings of this study are that human fetal heart-derived c-kit⁺ cardiac stem cells attenuate cardiac remodeling, decrease infarct size, improve left ventricular function, and differentiate into blood vessels and myocardium with greater potency than bone marrow-derived MSCs. MSCs also exert cardio-reparative effects, but a 30-fold greater cell number had less capacity for engraftment and differentiation than CSCs [31]. Together these findings offer key new insights into the cellular characteristics underlying successful cell-based cardiac repair.

Our study was conducted in the context of a growing body of evidence that stem cells may be used as a therapy to repair the

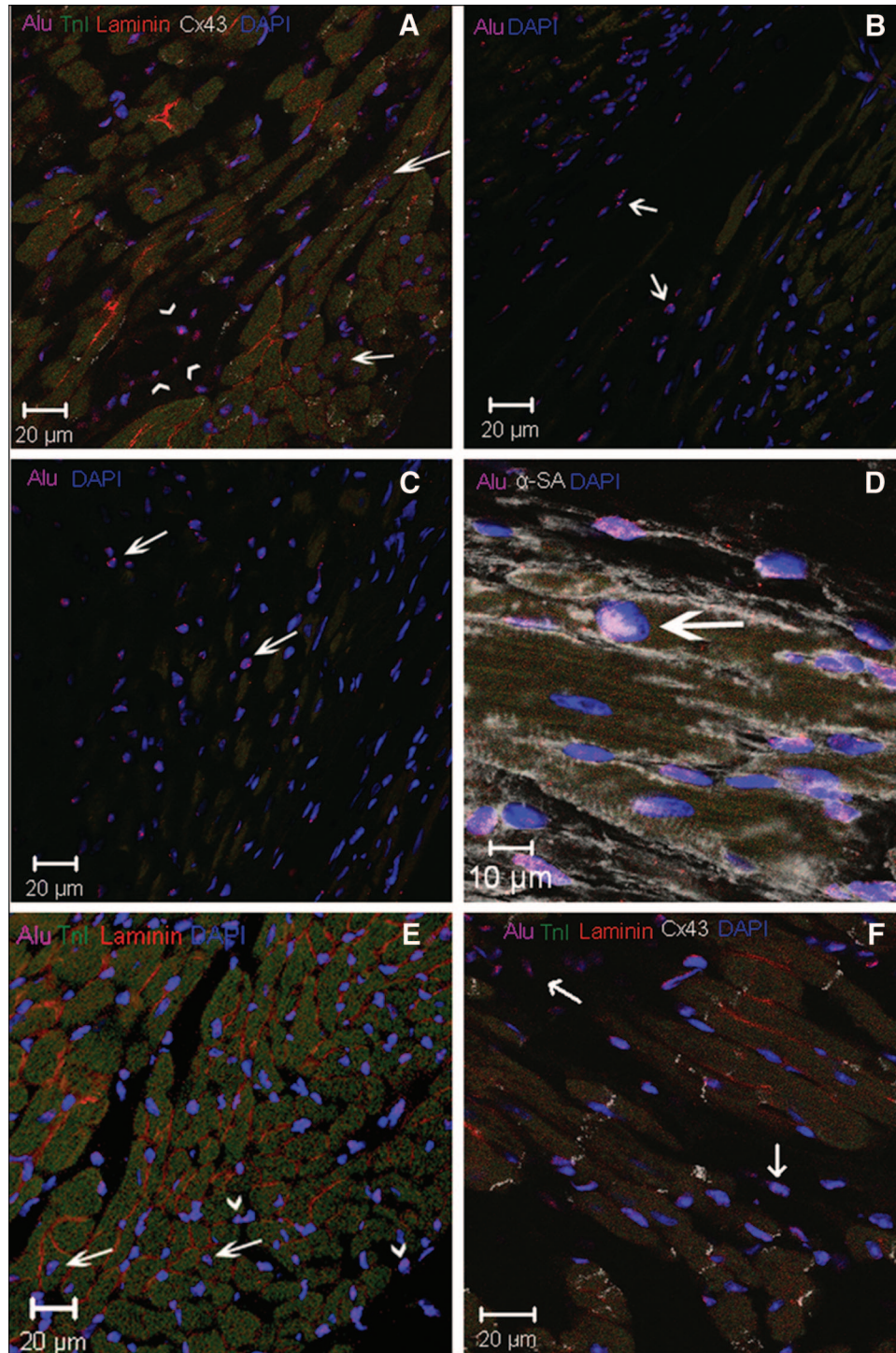


Figure 4. Cardiac stem cell-injected hearts ($\times 40$). **(A)**: Remote area: Alu (pink), Cx43 (white), Laminin (green), TnI (long arrow). **(B), (C)**: Alu⁺ cells in the **(B)** peri-infarct area and **(C)** infarcted area. **(D)**: Border zone: Alu (pink), α SA, (white), coexpression (arrow). **(E)**: Remote area: Alu and TnI costaining (long arrow), laminin (orange). Intercellular space: Alu⁺ (short arrows). **(F)**: Border zone. Abbreviations: Cx43, connexin 43; DAPI, 4',6-diamidino-2-phenylindole; TnI, troponin I.

detrimental effects that result from MI. Currently, there is enormous interest in cell-based cardiac repair, and a major effort is being expended to identify the optimal cell sources. In this regard, experimental studies and clinical trials have used the gamut of cells, ranging from autologous whole bone marrow [32] to pluripotent stem cells [33, 34]. Two very promising avenues of work are currently testing the hypothesis that bone marrow-

derived MSCs or cardiac-derived stem cells have the capacity for engraftment within the heart and the ability to differentiate into the cellular elements that have been lost to injury. As yet, no stem cell therapy has succeeded in fully replacing scarred or injured myocardium with new contractile and perfused contractile myocardium. As we continue to strive toward that goal, one of the major gaps in our knowledge derives from an absence of a

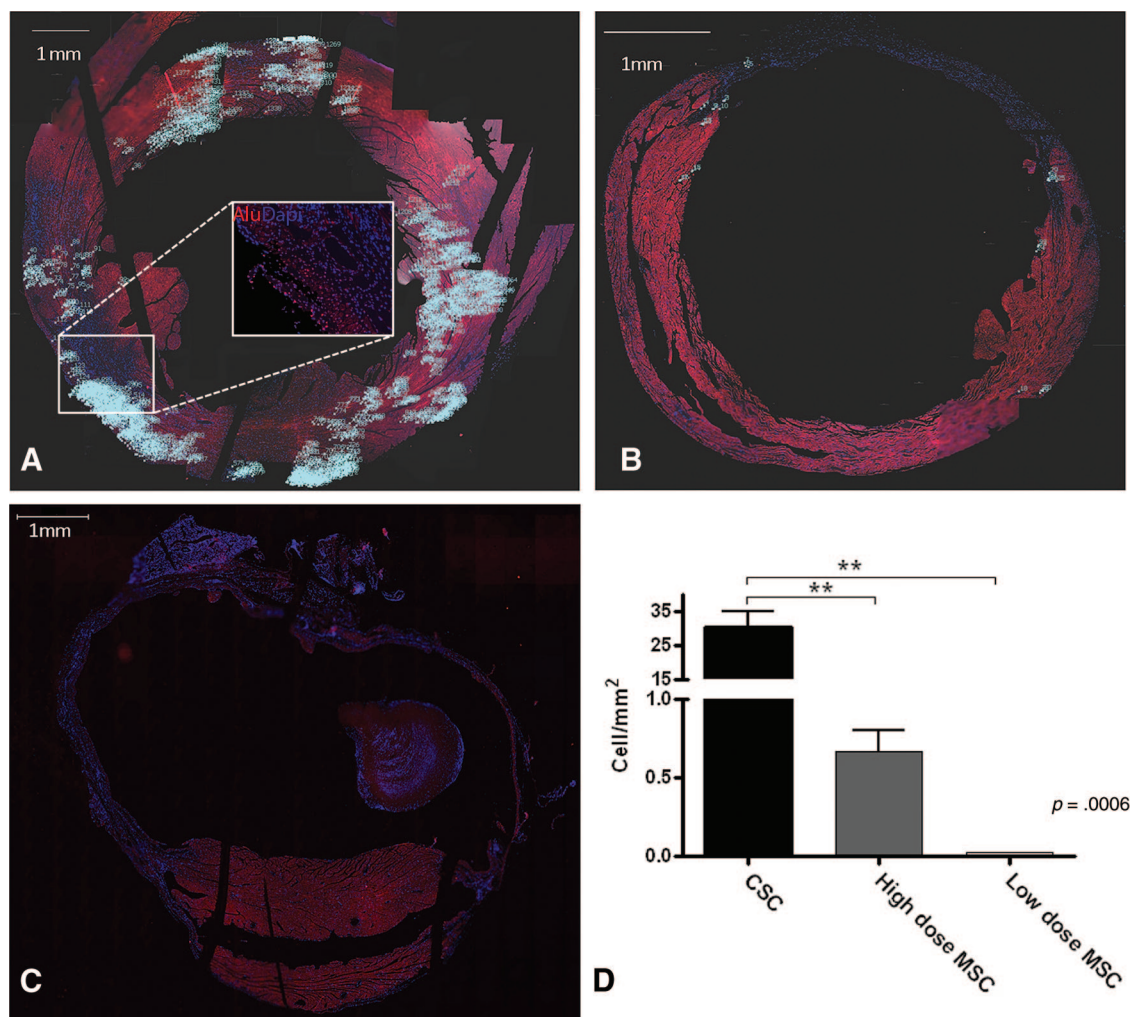


Figure 5. Human CSCs engraft better and are more widely distributed than human MSCs. Reconstructed images after tiling fluorescent scans at the midventricular level of Alu-stained hearts 8 weeks after cell delivery. Numbers = count of Alu⁺ cells. **(A):** CSCs, box outlines region of inset. Inset: an example of Alu⁺ cells (pink-stained nuclei) that were counted. **(B):** High-dose and **(C):** low-dose MSCs. DAPI (blue-stained nuclei). **(D):** Quantitation of Alu⁺ cells ($p = .0006$). **, $p < .01$. Abbreviations: CSC, cardiac stem cell; DAPI, 4',6-diamidino-2-phenylindole; MSC, mesenchymal stem cell.

direct comparison of various cell preparations to stimulate cardiac repair and for their capacity to engraft and differentiate into cardiac myocytes and vascular elements.

Beltrami et al. first reported the existence of c-kit-positive cardiac stem cells in 2003 [35]. CD 117 (c-kit) is a tyrosine kinase receptor for stem cell factor. Although c-kit is normally present on hematopoietic stem cells and neonatal cardiac cells [34], it can also be found, albeit in lower numbers, in adult cardiac tissue, where these cells retain their properties of stemness [35, 36, 37]. Cardiac c-kit⁺ cells have been identified in the mammalian heart, including that of humans, and are self-renewing; are capable of differentiation into myocytes, vascular smooth muscle, and endothelium; and are clonogenic [36, 37].

Both c-kit⁺ CSC and bone marrow-derived MSCs have entered clinical trials, supported by preclinical studies showing their promise for cardiac regeneration therapy [19, 20, 22]. Although there are numerous independent studies for these cells, they have never been compared directly for efficacy and cardiac regeneration in humans. MSCs are amplified from bone marrow based upon simple adherence to culture dishes and the capacity to grow in culture [38]. These cells have the capacity to differen-

tiate into mesodermal lineages but exert other effects that contribute to cardiac repair, including trilineage cell differentiation, the production of cytokines and growth factors that promote repair, antifibrotic effects, immunomodulation, and, perhaps most importantly, the induction of endogenous stem cell activity [23, 39, 40]. Several preclinical studies with MSCs have shown improved cardiac function after MI [23, 30, 39], with the totality of evidence supporting a favorable impact and ability to both prevent and reverse ventricular remodeling. MSCs have been shown to be safe and provisionally effective in offsetting cardiac remodeling in a phase I clinical trial [27]. Some studies (conducted mainly in rodents with chronic arterial occlusions) have shown either no functional improvement [29] or functional improvement with limited or no cellular differentiation [39], leading to the conclusion that paracrine and vasculogenic effect represent predominant mechanisms.

Based on previous experiments with c-kit⁺ CSCs [35, 36, 37, 41], we hypothesized that, in a dose-matched experiment, c-kit⁺ cells from heart would be more potent than MSCs in repairing cardiac damage after ischemic injury. Previous studies have suggested that the cardiomyogenic potential of animal- and

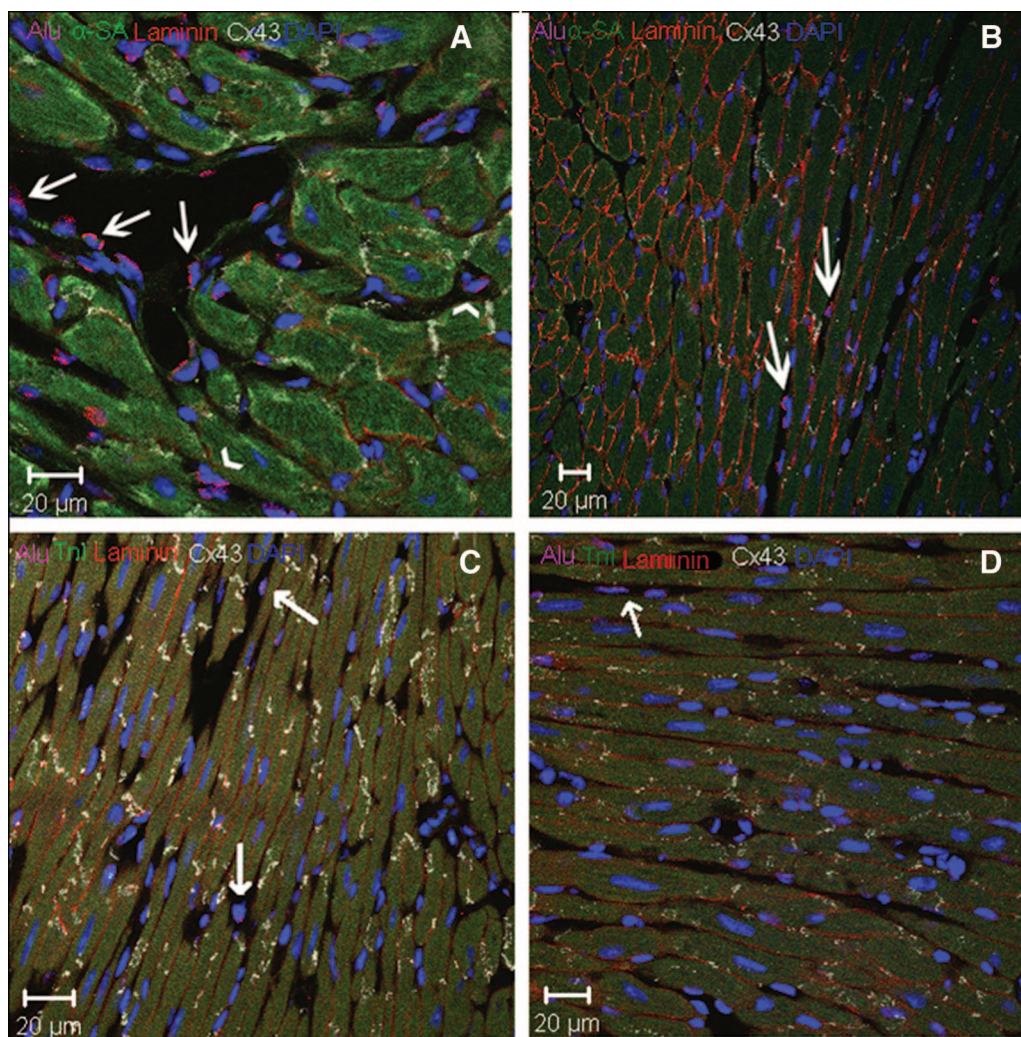


Figure 6. Immunohistochemistry of mesenchymal stem cell (MSC)-injected hearts, (A), (B): High-dose MSC. A: Border/peri-infarct zone: Alu⁺ cells (pink) in proximity to the vascular wall (white arrows) and between myocardiocytes (arrow head). (B): Remote zone: α -SA (green), Laminin (orange). (C), (D): 36×10^3 MSCs: Few Alu- and no Tnl-positive cells (arrows).

human-derived c-kit⁺ cells is greater both in vitro [18, 36, 42–44] and in vivo [17, 18]. Most studies have focused on injecting cultured cells into animals and have described the potential of these cells to differentiate into various elements of cardiac tissue [23, 25, 29, 39]. However, passaging c-kit⁺ cells potentially alters c-kit on the cell surface [28]. To surmount this problem, our study has, for the first time, carried out a direct comparison of the cardioprotective effects of fresh c-kit⁺ CSCs and cultured MSCs in an in vivo, ischemic, murine model. Furthermore, we compared these CSCs against both an equivalent (low-dose) and an approximately 30-fold excess (high-dose) number of MSCs. To address the issue of adult CSCs, we performed experiments using cultured adult c-kit⁺ CSCs; these cells, although not as potent as the fetal CSCs, still retained an efficacy achieving a similar amelioration of EF decline as did the high-dose MSCs. Importantly, this improvement was achieved using 30-fold lower cell concentration, illustrating the greater potency CSCs compared with MSCs.

After a relatively long-term follow-up (8 weeks), we identified human CSCs and MSCs in the heart by their staining for Alu-sequences. Surviving CSCs and MSCs from the high-dose group were embedded in the myocardium and appeared to be

associated with vascular structures. However, only the CSCs differentiated into cardiomyocytes (Figs. 4, 5). In the low-dose MSC group, few cells were found embedded in the myocardium after 8 weeks, and no cells were seen in vascular walls. Quantification of Alu⁺ cells showed that CSCs have high survival and engraftment capacity in the toxic ischemic environment. Furthermore, they have a greater ability than MSCs to differentiate into cardiac elements, despite the relatively low number of CSCs that were injected. In addition, our results suggest that the ability of MSCs to elicit repair may depend on the dose of cells injected and may help explain the minimal improvements and inconsistencies in clinical trials using MSCs [4]. We found that 1×10^6 MSCs was an effective dose when injected into a mouse heart that normally has $\sim 8 \times 10^6$ cells [45]. An equivalent dose for humans would be $\sim 325 \times 10^6$ MSCs, since the human heart has $\sim 2.6 \times 10^9$ cells [45], which is somewhat higher, but of the order of magnitude currently used in ongoing clinical trials [46].

Importantly, we have previously described myocyte differentiation of MSCs in large animals using a reperfusion model [30, 47]. These animals have different hemodynamic characteristics than do rodents, and these in vivo experiments suggest that mechanical loading and tissue perfusion are crucial for myocyte

differentiation of MSCs to occur. Thus, the lack of myocyte differentiation by MSCs in our mouse model suggests important differences in lineage differentiation between large and small animal models. Despite this limitation, it is clear that CSCs are superior in this regard and point to their potential advantages over MSCs to promote repair following ischemic heart damage. Furthermore, they are effective at a surprisingly low dose/efficacy ratio. Another significant aspect of this study is that we have used human cells, the candidate cells for clinical application. We avoided using fluorescent labeling, considering that green fluorescent protein labeling impairs actin myosin interaction [48].

Cultured MSCs are the typical cell source in most animal studies and clinical trials and represent a model against which all other cell types can be compared. They represent a (relatively) pure population of cells and are readily available for clinical use when needed. However, a high number of passages or time in culture might affect their protective effect [49] and might explain, in part, the inferiority of MSCs in this study compared with fresh CSCs. It is also important to note that there are various benefits of using MSCs. MSCs clearly have efficacy [30, 47], although more cells are required than CSCs, and MSCs can also be used as an allogeneic graft. Perhaps most importantly, we have previously shown that MSCs stimulate endogenous c-kit⁺ CSC proliferation and differentiation [30], and this stimulation may represent a crucial mechanism underlying MSC-mediated repair. This mechanism raises the intriguing hypothesis that the combination of MSCs and c-kit⁺ CSCs may yield a highly potent therapeutic regimen [20].

Limitation

The potential limitation to this study is that we used fetal hearts in order to have a source of cells containing more tissue than an adult heart biopsy sample. Fetal hearts are not clinically applicable as a stem cell source, and they potentially differ from adult CSCs in their differentiation capacity [50]. However, we also used cultured adult cardiac stem cells in additional experiments and revealed similar efficacy to the high concentration of MSCs.

CONCLUSION

In summary, in a direct comparison, we have shown that human CSCs expressing c-kit have an enhanced cardiopoietic potential relative to human bone marrow-derived MSCs. Our findings help define the parameters around the usage of CSCs and MSCs for cardiac indications with regard to dosing and mechanism of action, and the present data support the ongoing rigorous testing of both CSCs and MSCs for their role in the armamentarium of cells that could be used in cell-based therapy for a variety of cardiac conditions, including ischemic heart disease and heart failure.

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AUTHOR CONTRIBUTIONS

B.N.O.: conception and design, collection and/or assembly of data, data analysis and interpretation, manuscript writing; G.L., C.J., A.V.T., S.L., J.D.S., K.H., and M.D.: collection and/or assembly of data; W.B.: data analysis and interpretation, manuscript writing; I.M.: conception and design, provision of study material; J.M.H.: conception and design, data analysis and interpretation, manuscript writing, financial support, final approval of manuscript.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors indicate no potential conflicts of interest.

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