Heart Failure

Patient Characteristics and Cell Source Determine the Number of Isolated Human Cardiac Progenitor Cells

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Background—The identification and isolation of human cardiac progenitor cells (hCPCs) offer new approaches for myocardial regeneration and repair. Still, the optimal source of human cardiac progenitor cells and the influence of patient characteristics on their number remain unclear. Using a novel method to isolate human cardiac progenitor cells, we aimed to define the optimal source and association between their number and patient characteristics.

Methods and Results—We developed a novel isolation method that produced viable cells (7×10^6±6.53×10^5/g) from various tissue samples obtained during heart surgery or endomyocardial biopsies (113 samples from 94 patients 23 to 80 years of age). The isolated cardiac cells were grown in culture with a stem cell expansion medium. According to fluorescence-activated cell sorting analysis, cultured cells derived from the right atrium generated higher amounts of c-kit+ (24±2.5%) and Islet-1+ cells (7%) in culture (mean of passages 1, 2, and 3) than did cultured cells from the left atrium (7.3±3.5%), right ventricle (4.1±1.6%), and left ventricle (9.7±3%; P=0.001). According to multivariable analysis, the right atrium as the cell source and female sex were associated with a higher number of c-kit+ cells. There was no overlap between c-kit+ Islet-1 expression. In vitro assays of differentiation into osteoblasts, adipocytes, and myogenic lineage showed that the isolated human cardiac progenitor cells were multipotent. Finally, the cells were transplanted into infarcted myocardium of rats and generated myocardial grafts.

Conclusion—Our results show that the right atrium is the best source for c-kit+ and Islet-1 progenitors, with higher percentages of c-kit+ cells being produced by women. (Circulation. 2009;120:2559-2566.)

Key Words: atrium ■ cells ■ heart failure ■ stem cells

The isolation and expansion of human cardiac stem cells offer new therapies for myocardial regeneration and repair. Cells could be expanded in vitro, providing a large number of human cardiac progenitor cells (hCPCs) for therapeutic applications. Nevertheless, despite significant advances and promising findings in animals, several issues have not yet been thoroughly investigated. First, an efficient approach is needed to isolate hCPCs from the human heart. Current methods are derived mostly from rodents, with only variable efficiency, thereby limiting the potential clinical application of hCPCs. Second, although hCPCs can be isolated from different sections of the heart, such as the septum and atrial appendages, correlation between the source of the sample and the number of hCPCs has not yet been determined. Finally, although it has been reported that the number of hCPCs increases during heart disease, it is unclear whether hCPCs could be isolated and grown from all patients.

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2559
Figure 1. Source of tissue samples. A total of 113 myocardial tissue samples from 94 patients were collected. The first 56 samples were used to develop and optimize the cell isolation protocol. To minimize intersample variability, FACS analysis was conducted on the subsequent 57 specimens (from 40 patients). All 57 specimens were isolated and grown in the same conditions and analyzed by FACS with c-kit antibody from the same batch. Of these, 4 patients with end-stage heart disease made up a different subgroup and were analyzed separately. Two patients donated 2 samples from different areas. Three patients donated 2 samples from different areas, and 1 patient donated 3 samples from different areas. Four patients donated 4 samples from different areas.

Patients and Tissue Samples

A total of 113 myocardial tissue samples from 94 patients were collected from discarded tissues that were excised as routine procedure during open heart surgery, heart transplantation, or endomyocardial biopsies. Myocardial specimens (0.1 to 4.5 g) were obtained from the right or left atrial appendages, right ventricle, or left ventricle (LV).

Figure 1 shows the sources of tissue samples. The first 54 patients donated 56 samples (52 patients contributed 1 tissue sample, 2 patients contributed 2 samples from different areas) that were used to develop and scale up the cell isolation, expansion, and fluorescence-activated cell sorting (FACS) analysis protocols. To minimize intersample variability, FACS analysis was preformed on the subsequent 57 specimens (from 40 patients). All 57 specimens were isolated and grown in the same conditions and analyzed by FACS with c-kit antibody from the same batch. Of these, 4 patients with end-stage heart disease made up a different subgroup and were analyzed separately. Two patients donated 2 samples from different areas. Three patients donated 2 samples from different areas, and 1 patient donated 3 samples from different areas. Four patients donated 4 samples from different areas.

The following primary antibodies were used: c-kit (Dako, Glostrup, Copenhagen, Denmark), Islet-1 (R&D Systems, Minneapolis, Minn), GATA-4 (R&D Systems), cardiac α-actin (Acris), Ki-67 (Dako), collagen type 1 (Chemicon International, Temecula, Calif), CD31 (Dako), tryptase (Dako), CD45 (Dako), and CD68 (Dako). Secondary antibodies included the following: FITC donkey anti-mouse IgG (Chemicon), Cy3 donkey anti-rabbit IgG (Jackson Laboratory, Bar Harbor, Me), Cy3 goat anti-rat (Jackson), Cy2 rabbit anti-goat (Jackson), and Cy5 goat anti-mouse (R&D Systems). C-kit expression levels (mean ± SEM) of living cells were calculated after the first, second, and third passages.

In Vitro Differentiation Assays

In vitro assays for differentiation into osteoblasts and adipocytes and toward myogenic lineage examined multipotential differentiation capabilities of hCPCs as previously described.7 For osteogenic differentiation, cells were seeded at 2 × 10^4/cm^2 in DMEM (Biological Industries) with 10 mmol/L β-glycerophosphate (Sigma), 10^−7 mol/L dexamethasone (Sigma), and 0.2 mmol/L ascorbic acid (Sigma). Cultures were stained with alizarin red for identification of differentiated cells. For adipogenic differentiation, cells were cultured at 2 × 10^4/cm^2 with 10% horse serum (Biological Industries) and 100 ng/mL insulin. Cultures were maintained for 14 days with medium exchanges every 3 to 4 days. Lipid depositions were examined with Oil Red O staining (Sigma). For myogenic differentiation, cells were plated at 2 × 10^4/cm^2 and treated with 10 μmol/L 5-azacytidine (Sigma) in DMEM containing 10% FBS (Biological Industries) for 24 hours. After this procedure, cells were maintained in 2% FBS medium without 5-azacytidine for 2 weeks. Cultures were stained for cardiac troponin I and sarcomeric actin for assessment of differentiation.

Cell Transplantation

The injected cells were pooled from 2 to 3 different patient cultures. A magnet-activated cell sorter (Miltenyi MACS, Miltenyi Biotec, Bergisch Gladbach, Germany) with antibodies against human c-kit was used for cell culture enrichment before cell transplantation (in 1 group). Overall, 52 male athymic nude rats (~200 g; Harlan Laboratories, Indianapolis, Ind) were included in our cell transplantation studies. First, we injected 1 × 10^6 c-kit+–enriched cells into the myocardium of 2 noninfarcted athymic nude rats. In the next experiment, cells were injected into a rat model of myocardial infarction as previously described.8,9 Briefly, 50 rats were subjected to myocardial infarction by permanent proximal left coronary artery occlusion. The rate of perioperative mortality was 30%. Seven days after myocardial infarction, the surviving animals (n=35) were randomized for a single injection into the infarcted myocardium of 1 × 10^6 unselected cultured cells (n=12), saline (n=11), or c-kit+–enriched cells (n=12). Animals were monitored for 1 month.
One month after transplantation, RNA was extracted (5prime, Hamburg, Germany) from rat hearts, and reverse-transcription polymerase chain reaction (PCR) was done with the Verso cDNA kit (Applied Biosystems cDNA Kit, Foster, Calif) according to the manufacturer’s instructions. For real-time PCR, the levels of expression of human GATA4, human HPRT1, and rat β-actin were compared by use of a Taqman real-time PCR assay on demand according to standard procedure. Real-time PCR was done with the ABI PRISM 7900 Real Time System and analyzed with SDS 2.1 software (Applied Biosystems).

Histological and Immunohistochemical Examinations
For histology studies, animals that had received transplants were euthanized at 1 week (n=2) or 1 month (n=35) after cell transplantation. The hearts were perfused with 10% buffered formalin and sectioned into 3 transverse slices parallel to the atrioventricular ring. Hearts were fixed using 2 different methods for various antibody types. For paraffin-embedded sections, each slice was fixed with 10% buffered formalin, embedded in paraffin, and sectioned into 5-μm slices. Serial sections were stained for immunofluorescent staining with DAPI (nuclear staining) and immunolabeled with antibodies against human mitochondria (Chemicon), human cardiac actin (Acris), connexin 43 (Sigma), c-kit (Dako), CD31 (Dako), and ED1 (Serotec). For frozen sections, each slice was fixed with 30% sucrose, and representative sections were embedded in optimal-cutting-temperature embedding compound fixative and snap-frozen. Frozen sections (5 μm) were used for immunofluorescent staining with anti-human cardiac actin (Acris) and DAPI.

Statistical Methods
Statistical analyses were carried out with SPSS 15.01 software (SPSS Inc, Chicago, Ill). Demographic characteristics, clinical characteristics, and percentage of c-kit+ cells were compared by use of independent t tests and Fisher exact test for continuous and categorical measurements, respectively. The percentage of c-kit+ cells in culture was calculated from the mean of cell passages 1, 2, and 3. To determine the association between percentage of c-kit+ cells and the anatomic origin of tissue sample, we used a mixed-effects model on the tissue sample–based data from patients without end-stage heart failure. Subsequently, to determine the association between percentage of c-kit+ cells and clinical characteristics, we analyzed data from patients without end-stage heart failure. A multivariable linear regression with stepwise selection procedure was applied. The model included sex, age, source of tissue, smoking, hypertension, and LV ejection fraction. The R2 of the model was 0.65, suggesting that the model explains 65% of the variability. Significance level was defined as α=0.05. No imputations for missing values were applied.

All authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results
C-Kit Cells Can Be Isolated From Different Patients
Figure 1 shows the sources of tissue specimens. With the use of a novel isolation method, all 113 tissue samples yielded high numbers of viable cells (7×106±6.53×105 cells per g sample) from all 4 areas within the heart. There were no differences in clinical characteristics between patients who donated tissues for the scale-up protocol and patients with heart disease who donated tissues for the detailed FACS analysis (Table I of the online-only Data Supplement).

Reproducible and constant FACS analysis for characteristics of c-kit expression was carried out on 57 specimens (40 patients with heart disease and end-stage heart failure), and the data were used for further analysis. Tissue specimens were taken during various types of cardiac interventions and from all 4 heart chambers (Table 1). Mean patient age was 61±12 years; 72% were male; 46% had hypertension; 29% had diabetes mellitus; and 50% had coronary artery disease (Table 2).

Isolated hCPCs Are Multipotent and Express Stem Cell Markers
A significant amount of the cardiac cultured cells expressed stem cell markers such as c-kit (22±2.5%) and Islet-1 (7%), early myogenic transcription factor GATA 4 (60±6%), and the sarcomeric cardiac protein α-actin (60±11%) (Figure 2). Costaining with c-kit and Islet-1 antibodies revealed that the c-kit– and Islet-1–positive cells were 2 distinct populations without overlap (Figure I of the online-only Data Supplement). Two other subpopulations were also found in the culture: c-kit+ hCPCs that were positive for GATA4 and cells that were positive for GATA4 and negative for c-kit. Cultures were not contaminated by other cells such as macrophages, fibroblasts, or hematopoietic, mast, or endothelial cells (<1%) (Figure 2). In vitro assays of differentiation into osteoblasts and adipocytes and toward myogenic lineage confirmed that these cells are multipotent. The cultures derived from the right atrium showed more differentiated cells (Figure 3C and 3G) than did cells from the left atrium (Figure 3E and 3I).

hCPCs Differentiate Into Myocardial Graft in the Infarcted Rat Heart
At 1 week after injection into normal myocardium, microscopic examination identified implanted progenitor cells that stained positive for c-kit (Figure 4A) and human cardiac α-actin with early sarcomere formation (Figure 4B). Furthermore, 1 month
The percentage of c-kit+ cells was similar in both sexes, in smokers and nonsmokers, in patients with and without LV hypertrophy, and in those presenting with pulmonary hypertension, valvular disease, and atrial dilatation on echocardiography (Table 3). No correlation was found between medical therapy, including aspirin, angiotensin-converting enzyme inhibitors, amiodarone, and statins, and the number of c-kit+ cells.

Patients with end-stage heart failure (n=4) with a mean ejection fraction of 15±5% who underwent heart transplantation made up a separate group (Figure 1 and Table I of the online-only Data Supplement). In patients with end-stage heart failure, c-kit percentages in cell cultures were higher than in other patients (31±4% versus 17±2%, respectively; P<0.001), with the highest number derived from the LV, in contrast to right atrial predominance in patients without end-stage heart failure.

In multivariable analysis with multiple predictors and a stepwise selection procedure, sex emerged as a characteristic strongly related to c-kit+ once tissue source (right atrium) was taken into account (Table 4). The number of c-kit+ cells produced from the right atrial tissue was significantly higher than obtained from other sources (βsource=19.1±3.1; P<0.001). Furthermore, the number of c-kit+ cells was significantly higher in cultures derived from women than those derived from men (βsex=12.2±3.4; P<0.001).

**Discussion**

The present study is the first systematic attempt to correlate clinical characteristics with the number of hCPCs. The main findings of the present study suggest for the first time that the right atrium is the best source for c-kit and Islet-1 hCPCs and that women produce higher amounts of c-kit+ cells. In addition, we present a novel, efficient, and reproducible method to isolate c-kit+ hCPCs from diverse tissue samples. Compared with previous reports,3,10–14 the present isolation protocol has a nearly 100% success rate in isolating hCPCs (Table II of the online-only Data Supplement), which suggests that hCPC isolation is feasible for a wide range of cardiac patients.

**Comparison With Previous Reports**

The anatomic source of hCPCs in the heart is important in our understanding of their role in maintaining cardiac function and therapeutic potential. Pouly et al15 performed a histopathological study analyzing tissue samples from biopsies of a right-sided septum from heart transplant patients and right atrial appendage specimens from patients with ischemic cardiomyopathy. They found higher amounts of c-kit+ cells in septal biopsies than in the right atrial appendage. However, the septal biopsies were taken from heart transplant patients, a third of whom had some degree of rejection and were on immunosuppressive therapy. In the present study, we compared cell cultures from tissues derived from various patients. When analyzing data from a number of patients, we incorporated multivariable linear regression to eliminate potential differences in baseline characteristics.

Two new findings in our study are related to Islet-1+ cells and could extend our knowledge of postnatal hCPCs. Islet-1 identifies the second heart field lineage cells that are added to the developing heart tube and give rise to the outflow tract.
the right ventricular region, and the main parts of the atrial tissue.\(^{16,17}\) In our study, specimens from the right atrial appendage generated the highest amounts of Islet-1 cells. This finding matches our earlier finding that the right atrium is the best source of c-kit cells. However, there was no overlap between c-kit and Islet-1 expression on the cultured cells, and they represent 2 different cell populations. These findings could be relevant to the potential clinical application of our study and to the development of regenerative stem cell therapies for heart disease.

**Figure 2.** Flow cytometry analysis of cultured cells shows stem and progenitor markers with no contamination with fibroblasts, macrophages, or endothelial or mast cells. Viable cells were produced from various tissue samples obtained during heart surgery or endomyocardial biopsies. After digestion with an enzymatic cocktail, cells were cultured in an expansion medium and passaged every 5 days. Subsequent analyses were done on the entire population of cultured cells. Flow cytometry analysis was used for assessment of hCPC percentage in the derived cells. After 3 passages, cells were removed from the plate and incubated with both primary and secondary antibodies and resuspended in FACS buffer. Flow cytometry analysis with specific antibodies confirmed the presence of hCPCs with stem cell markers such as c-kit (A) and Islet-1 (Isl-1; B), proliferation marker Ki-67 (C), cardiomyogenic transcription factor GATA-4 (D), and myogenic proteins such as cardiac \(\alpha\)-actin (E). In addition, the flow cytometry analysis verified no contamination of the cultured cells by macrophages (CD68; F), fibroblasts (collagen type 1; G), hematopoietic cells (CD45; H), endothelial cells (CD31; I), or mast cells (tryptase; J) (all <1% of the cultured cells).

**Patient Characteristics and the Number of hCPCs**

One of the most interesting findings in the present study is that female sex is associated with a higher number of c-kit\(^+\) cells. Although the explanation for this finding is unclear, it is consistent with previous reports that suggested variability in stem cell function and numbers between the sexes.\(^{18--20}\)

Another relevant observation is the inverse relationship between end-stage heart failure and the number of c-kit\(^+\) cells. Although we examined only 4 hearts with end-stage
heart failure, previous reports also correlated increased numbers of CPCs in humans with significant aortic stenosis, acute and chronic ischemia, and other cardiomyopathies.\textsuperscript{1,6,21–23} Together, these findings support the notion that the endogenous reparative system is stimulated in the injured heart.\textsuperscript{24} A recent report has suggested that c-kit\textsuperscript{+} cells have increased proliferative capacity when cultured with extracellular matrix obtained from a failing heart.\textsuperscript{25} Thus, local signals that stimulate CPC response to injury could explain our findings.

Previous fundamental work on stem cell aging in the adult heart has indicated that the cardiac progenitor population undergoes an aging process that could be attributed to either telomere shortening–mediated cell senescence or cumulative cellular trauma.\textsuperscript{19,26,27} Consequently, histological analysis of myocardial tissue from elderly patients with cardiomyopathy (76 ± 4 years of age) showed lower numbers of c-kit\textsuperscript{+} cells compared with younger patients.\textsuperscript{28} Thus, one of the surprising findings of the present study was that age does not significantly affect the number of hCPCs derived from myocardial

**Figure 3.** In vitro differentiation assays indicate the multipotentiality of hCPCs. In vitro assays examined the multipotency of hCPCs by differentiation toward myogenic lineage or alternatively into osteoblasts or adipocytes. For myogenic differentiation, cells were treated with 5-azacytidine for 24 hours and maintained in 2% FBS medium for 2 weeks. Cardiomyogenic differentiation of hCPCs was confirmed by positive staining for cardiac troponin I and early sarcomere formation (A and B; arrowheads on typical striation). For adipogenic differentiation, cells were cultured with 10% horse serum and insulin for 14 days. Positive staining for Oil Red O confirms induced adipogenic differentiation of hCPCs derived from the right (C) and left (E) atria vs untreated hCPCs (D and F, respectively). For osteogenic differentiation, cells were seeded with β-glycerophosphate, dexamethasone, and ascorbic acid. Positive staining for alizarin red shows induced osteogenic differentiation of hCPCs derived from the right (G) and left (I) atria vs no differentiation in untreated hCPCs (H and J, respectively). Of note is that more differentiated cells in culture were derived from the right atrium (C and G) than the left atrium (E and I).

**Figure 4.** Histological analysis of graft cell survival and differentiation. hCPCs were injected into normal or infarcted hearts of nude rats. The injected cells were pooled from 2 to 3 different patient cultures. A magnet-activated cell sorter with antibodies against human c-kit was used for cell culture enrichment before cell transplantation. A and B. First, we injected $1 \times 10^6$ c-kit\textsuperscript{+}–enriched cells into the myocardium of 2 noninfarcted athymic nude rats. After 1 week, the hearts were fixed and embedded. Sections were stained with an antibody to c-kit and human fetal cardiac α-actin to identify hCPCs and specifically human cardiac graft cells. Histological analysis revealed implanted c-kit\textsuperscript{+} progenitor cells (A; white arrow) and early sarcomere formation accompanied by the typical striation and positive staining for human fetal cardiac α-actin (B; green, arrow). In the next experiment, $1 \times 10^6$ c-kit\textsuperscript{+}–enriched cells were injected into the infarcted heart. Seven days after myocardial infarction, animals were treated with a single injection of $1 \times 10^6$ c-kit\textsuperscript{+}–enriched cells into the infarcted myocardium. One month after transplantation, a representative image shows a well-organized myocardial cell graft expressing human cardiac α-actin (white arrow) and connexin 43 (C; red, arrow). Nuclei are stained blue with DAPI.
specimens. This finding is consistent with those of Anversa et al., who suggested that chronological age is not necessarily the main determinant of the functionality and frequency of hCPCs. However, other background conditions may play an important role in the physiology of hCPCs. An additional explanation could be that even in the aged heart, hCPCs are probably located in certain regions within the myocardium (eg, atrium) that retain significant amounts of telomerase activity or in situ stimulation.

**Table 3. Percentage of C-Kit–Positive Cells in Culture According to Patient Clinical Characteristics**

<table>
<thead>
<tr>
<th>C-Kit±SEM, %</th>
<th>Yes</th>
<th>No</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline characteristics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>22.5±6</td>
<td>16±2</td>
<td>0.3</td>
</tr>
<tr>
<td>Diabetes</td>
<td>20±5</td>
<td>17±2</td>
<td>0.5</td>
</tr>
<tr>
<td>Hypertension</td>
<td>23±3</td>
<td>13±3</td>
<td>0.02</td>
</tr>
<tr>
<td>Smoker</td>
<td>10±4</td>
<td>19±2</td>
<td>0.2</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>20±3</td>
<td>15±3</td>
<td>0.3</td>
</tr>
<tr>
<td>Ischemic heart disease</td>
<td>19±2</td>
<td>17±4</td>
<td>0.65</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>20±5</td>
<td>17±2</td>
<td>0.5</td>
</tr>
<tr>
<td><strong>Cardiac measurements by echocardiography</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Impaired LV function*</td>
<td>19±4</td>
<td>17±3</td>
<td>0.63</td>
</tr>
<tr>
<td>LV hypertrophy†</td>
<td>14±4</td>
<td>20±3</td>
<td>0.21</td>
</tr>
<tr>
<td>Dilated right atrium‡</td>
<td>18±3</td>
<td>19.5±7</td>
<td>0.85</td>
</tr>
<tr>
<td>Dilated left atrium†</td>
<td>18±2</td>
<td>18.6±3</td>
<td>0.97</td>
</tr>
<tr>
<td>Pulmonary hypertension§</td>
<td>14±4</td>
<td>19±3</td>
<td>0.27</td>
</tr>
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</table>

n=36. 
*Defined as LV ejection fraction ≤50%. 
†Defined as end-diastolic interventricular septum thickness ≥11 mm. 
‡Defined as atrial area ≥20 mm². 
§Defined as systolic pulmonary artery pressure >40 mm Hg.

**Table 4. Factors Associated With a Higher C-Kit Fraction in Culture by Stepwise Selection Procedure**

<table>
<thead>
<tr>
<th>Source of tissue</th>
<th>Coefficients</th>
<th>95% CI for β</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right atrium (n=22) vs other (n=14)</td>
<td>19.1</td>
<td>3.1 &lt;0.001</td>
</tr>
<tr>
<td>Sex</td>
<td>12.2</td>
<td>3.4 &lt;0.001</td>
</tr>
</tbody>
</table>

β indicates model coefficient estimators; 95% CI, confidence interval; and P, significance level for testing the hypothesis that the coefficients are 0. A model of linear regression with multiple predictors was applied to identify which predictors significantly contribute to high c-kit values. The following predictors were included: age, sex, source of tissue, impaired ejection fraction, smoking, and hypertension. A stepwise algorithm was applied to identify the most significant predictors of all predictors included in the model.

**Limitations**

We are aware of several limitations in our study. Various factors that might affect hCPC number and growth, such as the amount of extracellular matrix and tissue fibrosis, were not evaluated, thereby creating a possible bias with regard to the yield of cardiac progenitors. Additionally, the majority of samples were derived from different patients. Although interpatient variation was adjusted in a multivariable linear regression, it is an inherent bias that should be taken into consideration. Finally, functional assays such as activation, growth capabilities, clonability, and telomerase activity of hCPCs were not investigated, and their association with patient characteristics needs further research.

**Conclusions**

The findings of the present study suggest that it is feasible to isolate and expand c-kit or Islet-1 hCPCs from most patients undergoing heart surgery, with the right atrium being the best source for tissue specimens. We also show that women and patients with end-stage heart failure produce a higher percentage of c-kit+ cells. Our findings could enhance the development of novel approaches in regenerative cardiovascular medicine. Further research is needed to develop efficient methods to guide hCPCs to myogenic differentiation and to determine whether these cells could be used for replacement of injured myocardium by direct cell transplantation or in situ stimulation.

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Disclosures
None.

References

CLINICAL PERSPECTIVE
The isolation and expansion of autologous human cardiac progenitor cells may offer new therapies for myocardial regeneration and repair. The present study shows that human cardiac progenitor cells can be obtained from small, clinically relevant myocardial specimens that could be collected during any cardiac surgery or percutaneous myocardial biopsy. Furthermore, these cells can be isolated from a wide range of patients, including young and old patients, those with various comorbidities such as hypertension and diabetes mellitus, and those with diverse types and severity of heart disease. Our study suggests that the best source for cardiac progenitor cells is the right atrium and that these cells are more abundant in women. Our findings could enhance the development of novel approaches in regenerative cardiovascular medicine. These isolated cells could be expanded in vitro, thereby providing a large number of cells for therapeutic applications. Alternatively, if these progenitor cells could be stimulated and activated in situ, with certain growth factors or small molecules, they could provide a tool for myocardial regeneration and the treatment of patients with heart disease.
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Supplemental Tables

**Supplement Table 1:**

Characteristics of the initial 54 patients, whose samples were used for protocol development and scale up, the 36 patients without and 4 patients with end-stage heart failure included in the final analysis.

<table>
<thead>
<tr>
<th></th>
<th>Patients used for protocol scale up (n=54)</th>
<th>Patients with heart disease (n=36)</th>
<th>Patients with end-stage heart failure (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>54, 61.5±11.9</td>
<td>36, 62.5±13.6*</td>
<td>4, 29.5±5.26</td>
</tr>
<tr>
<td>Body mass index</td>
<td>45, 27.8±5.5</td>
<td>26, 27.9±4.6*</td>
<td>3, 22.6±1.38</td>
</tr>
<tr>
<td>Gender:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>34, 63.6</td>
<td>26, 72.2*</td>
<td>3, 75.0</td>
</tr>
<tr>
<td>Female</td>
<td>20, 36.4</td>
<td>10, 27.8*</td>
<td>1, 25.0</td>
</tr>
<tr>
<td>Ischemic heart disease</td>
<td>28, 52.8</td>
<td>17, 50.0*</td>
<td>0, 0</td>
</tr>
<tr>
<td>Diabetes</td>
<td>12, 22.6</td>
<td>10, 28.6*</td>
<td>0, 0</td>
</tr>
<tr>
<td>Hypertension</td>
<td>28, 52.8</td>
<td>17, 47.2*</td>
<td>0, 0</td>
</tr>
<tr>
<td>Smokers</td>
<td>8, 15.1</td>
<td>4, 11.4*</td>
<td>0, 0</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>29, 54.7</td>
<td>22, 61.1*</td>
<td>0, 0</td>
</tr>
<tr>
<td>Medications</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>27, 50.9</td>
<td>17, 50.0*</td>
<td>1, 25.0</td>
</tr>
<tr>
<td>Statins</td>
<td>29, 54.7</td>
<td>19, 55.9*</td>
<td>0, 0</td>
</tr>
<tr>
<td>Amiodarone</td>
<td>3, 5.7</td>
<td>5, 14.7*</td>
<td>0, 0</td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>23, 43.4</td>
<td>18, 52.9*</td>
<td>3, 75.0</td>
</tr>
<tr>
<td>Echocardiographic parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Impaired LV function (EF≤50)</td>
<td>11, 27.5</td>
<td>12, 33.3*</td>
<td>3, 100.0</td>
</tr>
<tr>
<td>Aortic valve replacement</td>
<td>14, 26.4</td>
<td>9, 25.7*</td>
<td>0, 0</td>
</tr>
<tr>
<td>Aortic stenosis</td>
<td>8, 21.6</td>
<td>10, 31.3*</td>
<td>0, 0</td>
</tr>
<tr>
<td>Mitral regurgitation</td>
<td>13, 36.1</td>
<td>16, 47.1*</td>
<td>1, 25.0</td>
</tr>
<tr>
<td>Mitral stenosis</td>
<td>6, 16.2</td>
<td>3, 9.4*</td>
<td>0, 0</td>
</tr>
</tbody>
</table>

ACE – angiotensin-converting enzymes, EF – Ejection fraction, LV – Left ventricle

* Patients used for scale up (n=54) versus patients without end stage heart failure (n=36) used for final analysis, p value >0.05;

* Defined as LV ejection fraction ≤ 50%,
**Supplement table 2:**

In order to evaluate various isolation protocols with respect to cells yield, the cells were isolated from tissues collected during open-heart surgery by different enzymatic digestion cocktails. Different methods and several concentrations were used on the samples as detailed in supplemental table 2.

Please note that several methods were used originally to obtain cells form rodents. This fact may explain our low yield, since we converted those methods to isolate cells from humane tissue.

**Table 2: Isolation methods that we used to dissociated human heart tissues**

<table>
<thead>
<tr>
<th>technique used</th>
<th>Percentage of tissues that yielded any progenitor cells</th>
<th>The ratio living/ dead cells</th>
<th>The amount of viable cells per gram tissue*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. collagenase II + HEPES buffer</td>
<td>Argentin <em>et al</em> [10]</td>
<td>No data</td>
<td>No data</td>
</tr>
<tr>
<td>3. collagenase</td>
<td>Bearzi <em>et al</em> [3]</td>
<td>8 of 12</td>
<td>No data</td>
</tr>
<tr>
<td>4. Explanted technique</td>
<td>Bearzi <em>et al</em> [3]</td>
<td>46 of 70</td>
<td>No data</td>
</tr>
<tr>
<td>5. Explanted technique</td>
<td>Smith <em>et al</em> [5]</td>
<td>69 of 70</td>
<td>No data</td>
</tr>
<tr>
<td>6. Collagenase B+ Collagenase D+Protease XIV</td>
<td>Zhou <em>et al</em> [14]</td>
<td>No data</td>
<td>50%</td>
</tr>
<tr>
<td>7. Trypsin 0.1%</td>
<td>Parker <em>et al</em> [12]</td>
<td>No data</td>
<td>No data</td>
</tr>
<tr>
<td>8. 0.1% trypsin, 0.1% collagenase, 0.025% DNAase</td>
<td>Parker <em>et al</em> [11]</td>
<td>No data</td>
<td>No data</td>
</tr>
<tr>
<td>9. trypsin-EDTA 0.25% and dispase II (Sigma)</td>
<td>Itzhaki-Alfia <em>et al</em></td>
<td>113 of 113 samples</td>
<td>95-98%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>7x10^5 ±6.53x10^5 per gram</td>
</tr>
</tbody>
</table>
Supplemental Figure 1:

- 11.03% c-kit$^+$ cells
- 0.01% double staining for c-kit and islet-1
- 9.84% Islet-1$^+$ cells
Legend for Supplemental Figure 1:

Co-staining with c-kit and Islet-1 antibodies revealed that the c-kit and Islet-1 positive cells were two distinct populations.

Example of flow cytometry analysis of cultured hCSCs obtained from the right atrium (passage 3) shows 11.03% of c-kit+ and 9.84% of islet-1+ cells. There is no overlap between the two populations.