Heart Failure

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

Ayelet Itzhaki-Alfia, MSc; Jonathan Leor, MD; Ehud Raanani, MD; Leonid Sternik, MD; Dan Spiegelstein, MD; Shiri Netser, MSc; Radka Holbova; Meirav Pevsner-Fischer, MSc; Jacob Lavee, MD; Israel M. Barbash, MD

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 (7×10⁶±6.53×10⁵/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⁻ and 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.¹–³ 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.⁴ 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.³,⁵ Second, although hCPCs can be isolated from different sections of the heart, such as the septum,⁵ and atrial appendages,² 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,¹,⁶ it is unclear whether hCPCs could be isolated and grown from all patients.

Received January 7, 2009; accepted September 21, 2009.

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The online-only Data Supplement is available with this article at http://circ.ahajournals.org/cgi/content/full/CIRCULATIONAHA.109.849588/DC1.

Patients signed a written consent form for tissue collection according to a protocol approved by the Internal Review Committee on Ethics of Human Investigation of the Sheba Medical Center. Animal studies were done in accordance with the guidelines of the Animal Care and Use Committee of the Sheba Medical Center, Tel-Aviv University, which conforms to the policies of the American Heart Association and the Guide for the Care and Use of Laboratory Animals.

Clinical Perspective on p 2566

Understanding the characteristics of hCPCs could contribute to better insight into their role in maintaining cardiac function and their therapeutic potential as cardiac progenitors for myocardial repair. Using a novel method to isolate hCPCs, we sought to identify the optimal cardiac source for hCPCs and to determine a possible association between their number and patient characteristics.

Methods

Patients signed a written consent form for tissue collection according to a protocol approved by the Internal Review Committee on Ethics of Human Investigation of the Sheba Medical Center. Animal studies were done in accordance with the guidelines of the Animal Care and Use Committee of the Sheba Medical Center, Tel-Aviv University, which conforms to the policies of the American Heart Association and the Guide for the Care and Use of Laboratory Animals.

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Circulation is available at http://circ.ahajournals.org

DOI: 10.1161/CIRCULATIONAHA.109.849588

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atrial dimensions, and the presence of pulmonary hypertension.

Fraction ejection fraction (impaired LV function was defined as ejection

Preoperative echocardiography data were collected and included LV

made up a different subgroup (as shown in Table I of the online-only

Data Supplement) and were analyzed separately (Figure 1).

intersample variability, FACS analysis was performed on the subse-

protocol. To minimize intersample variability, FACS analysis was

samples were used to develop and optimize the cell isolation

All 57 specimens were isolated and grown in the same condi-

control, and admission drug therapy.

The patients' medical histories were collected from hospital

Conditions and analyzed by FACS with c-kit antibody from the same

tissue sample, 3 donated 2 samples from different areas, 1 donated 3

samples were used to develop and optimize the cell isolation

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 a-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. 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. Briefly, 50 rats were subjected to myocardial infarction by permanent proximal left coronary artery occlusion. The rate of peri-procedural 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), tryptase (Dako), CD68 (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 1 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).

Culturing 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

<table>
<thead>
<tr>
<th>Type of procedure*</th>
<th>Coronary artery procedures</th>
<th>Valvular procedures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coronary artery bypass surgery</td>
<td>14 (40)</td>
<td>Coronary artery replacement</td>
</tr>
<tr>
<td>Mitral valve replacement</td>
<td>9 (26)</td>
<td></td>
</tr>
<tr>
<td>Mitral valve repair</td>
<td>2 (6)</td>
<td></td>
</tr>
<tr>
<td>Tricuspid valve repair</td>
<td>3 (9)</td>
<td></td>
</tr>
</tbody>
</table>

Other procedures
- Maze procedure: 9 (26)
- Myectomy: 4 (12)
- Implantation of ventricular assist device: 3 (8)
- Heart transplantation: 4 (12)
- Percutaneous procedures: 2 (6)
- Biopsy: 6 (11)

Origin of tissue samples (n = 57)
- Right atrium: 26 (46)
- Left atrium: 14 (25)
- Left ventricle: 11 (19)
- Right ventricle: 6 (11)

Values are n (%).
*More than 1 procedure was performed during some operations. For example, 1 patient had both coronary artery bypass grafting and Maze procedures during the same surgery; this patient donated only 1 sample.
out on 2 housekeeping genes. A comparison was made between after injection, real-time reverse-transcription PCR was carried
number of hCPCs that were retained within the rat heart 1 month
unselected cell population or saline. To evaluate and quantify the
genic differentiation was found among animals that received
lated disc structure (connexin 43) (Figure 4C). No cardiomyo-
cells in culture compared with normotensive patients
hypertension had a significantly higher percentage of c-kit
ventricle, and left atrium. As shown in Table 3, patients with
impaired multivariable linear regression to eliminate potential
Variables Associated With the Number of C-Kit+ Cells
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 com-
pared cell cultures from tissues derived from various patients.
When analyzing data from a number of patients, we incorpo-
ated disc structure (connexin 43) (Figure 4C). No cardiomyo-
cells into infarcted
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.

### Table 2. Clinical Characteristics of 36 Patients Included in the Multivariable Linear Regression Model

<table>
<thead>
<tr>
<th>Patient Characteristics</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y, mean±SD</td>
<td>36 (62.5±13.6)</td>
</tr>
<tr>
<td>Male sex</td>
<td>26 (72)</td>
</tr>
<tr>
<td>Body mass index, kg/m2, mean±SD</td>
<td>26 (28±5)</td>
</tr>
<tr>
<td>Cardiovascular risk factors, n (%)</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>17 (47)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>10 (29)</td>
</tr>
<tr>
<td>Smoking</td>
<td>4 (11)</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>22 (61)</td>
</tr>
<tr>
<td>Coronary artery disease, n (%)</td>
<td>17 (50)</td>
</tr>
<tr>
<td>Atrial fibrillation, n (%)</td>
<td>12 (33)</td>
</tr>
<tr>
<td>Medications, n (%)</td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>17 (50)</td>
</tr>
<tr>
<td>Statins</td>
<td>5 (15)</td>
</tr>
<tr>
<td>Amiodarone</td>
<td>19 (56)</td>
</tr>
<tr>
<td>Angiotensin-converting enzyme inhibitors</td>
<td>18 (53)</td>
</tr>
<tr>
<td>Echocardiographic data, n (%)</td>
<td></td>
</tr>
<tr>
<td>Impaired LV function*</td>
<td>12 (33)</td>
</tr>
<tr>
<td>LV hypertrophy†</td>
<td>12 (40)</td>
</tr>
<tr>
<td>Mitral valve stenosis</td>
<td>3 (9)</td>
</tr>
<tr>
<td>Mitral valve insufficiency</td>
<td>16 (47)</td>
</tr>
<tr>
<td>Aortic valve stenosis</td>
<td>10 (31)</td>
</tr>
<tr>
<td>Aortic valve insufficiency</td>
<td>2 (6)</td>
</tr>
<tr>
<td>Pulmonary hypertension‡</td>
<td>10 (31)</td>
</tr>
</tbody>
</table>

*Impaired LV function defined as LV ejection fraction ≤50%.
†LVH defined as end-diastolic interventricular septum thickness ≥11 mm.
‡Pulmonary hypertension defined as calculated systolic pulmonary artery pressure ≥40 mm Hg.

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 hyperten-
sion, 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.

The number of c-kit+ cells between the other areas, ie, LV, right
ventricle, and left atrium. As shown in Table 3, patients with
hypertension had a significantly higher percentage of c-kit+ cells in culture compared with normotensive patients
(23±3% versus 13±3%, respectively; P=0.02).
the right ventricular region, and the main parts of the atrial tissue. 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.

**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.

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. Together, these findings support the notion that the endogenous reparative system is stimulated in the injured heart. A recent report has suggested that c-kit+ cells have increased proliferative capacity when cultured with extracellular matrix obtained from a failing heart. 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. Consequently, histological analysis of myocardial tissue from elderly patients with cardiomyopathy (76±4 years of age) showed lower numbers of c-kit+ cells compared with younger patients. Thus, one of the surprising findings of the present study was that age does not significantly affect the number of hCPCs derived from myocardial...
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.

Conclusions
We thank Professor Dov Zipori from the Department of Molecular Cell Biology, Weizmann Institute of Science for his assistance with the differentiation protocols. We thank Dr Zohar Barbash from the Cancer Research Center at the Sheba Medical Center for her assistance during the real-time PCR experiments. We also thank Vivienne York for skillful editing of the manuscript and Zmira Silman for statistical analysis. This work was done as partial fulfillment of the requirements for a PhD degree for Ayelet Itzhaki-Alfia at Sackler Faculty of Medicine, Tel Aviv University, Israel.

Sources of Funding
We gratefully acknowledge support for this project provided by grants from the Legacy Heritage Fund of New York (Dr Leor); Tel-Aviv University PhD Student scholarship for stem cell research.
funded by the Legacy Heritage Fund; the Researcher-Physician Grant from the Israel Academy of Sciences and Humanities—The Bat Sheva de Rothschild Fund for the Advancement of Science in Israel (Dr Barbash); the Israel Ministry of Science, Culture, and Sport (Dr Leor); the Israel Science Foundation (Dr Leor); and the Seventh Framework Program, European Commission—Cardiovascular Disease (Dr Barbash and Leor).

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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Circulation. 2009;120:2559-2566; originally published online December 7, 2009;
doi: 10.1161/CIRCULATIONAHA.109.849588
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/120/25/2559

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http://circ.ahajournals.org/content/suppl/2010/01/19/CIRCULATIONAHA.109.849588.DC1

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(CIRCULATIONAHA/2009/849588)
**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>
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<th>Patients used for protocol scale up</th>
<th>Patients with heart disease</th>
<th>Patients with end-stage heart failure</th>
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<tr>
<td></td>
<td>n=54</td>
<td>n=36</td>
<td>n=4</td>
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<tr>
<td>Age (years)</td>
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<tr>
<td></td>
<td>61.5±11.9</td>
<td>62.5±13.6*</td>
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<tr>
<td>Body mass index</td>
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<td></td>
<td>27.8±5.5</td>
<td>27.9±4.6 *</td>
<td>22.6±1.38</td>
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<td>Gender:</td>
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<tr>
<td>Male</td>
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<tr>
<td></td>
<td>63.6</td>
<td>72.2*</td>
<td>75.0</td>
</tr>
<tr>
<td>Female</td>
<td>20</td>
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<td>1</td>
</tr>
<tr>
<td></td>
<td>36.4</td>
<td>27.8*</td>
<td>25.0</td>
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<td></td>
<td>52.8</td>
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<td>61.1*</td>
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<td>25.0</td>
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<td>14.7*</td>
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<td>3</td>
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<td>43.4</td>
<td>52.9*</td>
<td>75.0</td>
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<td>Echocardiographic parameters</td>
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<tr>
<td>Impaired LV function (EF≤50)*</td>
<td>11</td>
<td>12</td>
<td>3</td>
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<tr>
<td></td>
<td>27.5</td>
<td>33.3*</td>
<td>100.0</td>
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<td>Aortic valve replacement</td>
<td>14</td>
<td>9</td>
<td>0</td>
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<tr>
<td></td>
<td>26.4</td>
<td>25.7*</td>
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<td>21.6</td>
<td>31.3*</td>
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<td>47.1*</td>
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<td>16.2</td>
<td>9.4*</td>
<td>.0</td>
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</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;

a 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 from 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>
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<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>2. RDB proteolytic enzyme (Institute of Biology, Nes-Ziona, Israel).</td>
<td>Shneyvays <em>et al</em> 13</td>
<td>No data</td>
<td>No data</td>
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<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>
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<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>
</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.