"Human Cardiomyocyte Stem Cells as an alternative to cardiac tissue transplants"

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Introduction:

In the past few years it has been established that the heart contains a reservoir of Stem and Progenitor Cells. These Cells are positive for various stem/progenitor cell markers (c-kit, Sca-1, and Glycoprotein) properties. The adult human heart contains small populations of indigenous committed Cardiac Stem Cells (CSS) or multipotent Cardiac progenitor Cells, identified by their cell-surface expression of c-kit (the receptor for Stem cell factor), P-Glycoprotein (a member of the multidrug resistance protein family), and Sca-1 (stem cell antigen 1), a mouse hematopoietic Stem cell marker or a Sca-1-like protein.

Cardiac Stem Cells represent a logical source to exploit in cardiac regeneration therapy because, unlike other adult Stem Cells, they are likely to be intrinsically programmed to generate cardiac tissue in vitro and to increase cardiac tissue viability in vivo. Cardiac Stem cell therapy could, therefore, change the fundamental approach to the treatment of heart disease. Cellular Cardiomyoplasty may indeed be a novel method to regenerate damaged myocardium. Research has found the heart tissue cells, tissue samples, and embryonic stem cells from various sources of human myocardium and expanded them ex vivo by many folds without losing differentiation potential into Cardiomyocytes and vascular Cells, bringing autologous transplantation of CSSCs closer to clinical evaluation. Our method for ex vivo expansion of resident CSCs for subsequent autologous transplantation back into the heart, may give these cell populations, the resident and the transplanted one, the combined ability to Mediate myocardial regeneration to an appreciable degree, and may change the way in which cardiovascular diseases will be approached in the future.

Method:

Human Neonatal Atrium biopsy samples were collected from 5 females and 5 male patients at the time of surgery or during heart transplant. The biopsy samples were 60 – 135 mg, from age groups of 6 – 9 months, and the samples were stored in Celprogen’s human cardiac perfusion solution and shipped at 2-6°C to Celprogen for extraction of Stem Cells. The tissue samples were shipped within 24 hours, and upon receipt of the tissue samples Cardiac Stem Cells were extracted from the tissue utilizing Celprogen Cardiac Stem Cell Media and Extra-cellular Matrix.

The Cardiac Stem Cells were initially plated in T25 pre-coated flasks with Un-differentiation Extra-cellular Matrix and undifferentiation complete growth medium. After 12 hours of initial incubation 100% medium was replaced with 190% fresh pre-warmed medium. The suspension Cells were removed and transferred to another flask and the attached Cardiac Stem Cells were further cultured in 5% CO2 humidified incubator at 37°C temperature. After the initial 7 days in culture the Atrium Cardiac Stem Cells were subcultured and transferred to expansion Media and Matrix at a ratio of one T25 flask to 2 T75 flasks.

Once the Atrium Cardiac Stem Cells were expanded in culture conditions for 7 days, Cardiac Stem cell marker assays were performed on the cultured Cells with RT-PCR and immuno-histochemistry assays. Upon confirmation of the Atrium Cardiac Stem Cells they were then subjected to the Cardiac differentiation protocol for 7 days in culture in Differentiation Media and Matrix. Briefly, the Atrium Cardiac Stem Cells were cultured in the differentiation Media and Differentiation Matrix with regular changing of medium daily for 7 days. Upon differentiation of the Atrium Cardiac Stem Cells the cardiac tissue was further characterized for cardiac markers and cardiac gene, protein, Mitochondrial and cardiac profile.

Results:

The results are presented in figures 1-19 and graph 1. Briefly, figure 1 indicates the myogenic gene expressions of the following transcription factors: c-kit, Nkx2.5, GATA4, MEF2C, SERCA2a, MHC and Tropinin I of Human Atrium Cardiac Stem Cells.

**ACSC Differentiated Cardiomyocytes**

**Graph 1:** Atrium Cardiomyocyte Cells Differentiated in to Cardiomyocytes

**Figure 3:** Calcium profiles of Differentiated Cardiomyocytes from Human Atrium Cardiomyocyte Cells

**Figure 4:** Immunocytochemistry Characterization of Human Neonatal Atrium Cardiomyocytes

**Figure 5:** Calcium Profiles of Human Neonatal Atrium Cardiomyocytes Differentiated from Human Atrium Cardiomyocyte Cells

**Figure 6:** Calcium Profiles of Human Neonatal Atrium Cardiomyocytes Differentiated from Human Atrium Cardiomyocyte Cells

**Figure 7:** Calcium Profiles of Human Neonatal Atrium Cardiomyocytes Differentiated from Human Atrium Cardiomyocyte Cells

**Figure 8:** Calcium Profiles of Human Neonatal Atrium Cardiomyocytes Differentiated from Human Atrium Cardiomyocyte Cells

**Figure 9:** Calcium Profiles of Human Neonatal Atrium Cardiomyocytes Differentiated from Human Atrium Cardiomyocyte Cells

**Figure 10:** Calcium Profiles of Human Neonatal Atrium Cardiomyocytes Differentiated from Human Atrium Cardiomyocyte Cells

**Figure 11:** Calcium Profiles of Human Neonatal Atrium Cardiomyocytes Differentiated from Human Atrium Cardiomyocyte Cells

**Figure 12:** Calcium Profiles of Human Neonatal Atrium Cardiomyocytes Differentiated from Human Atrium Cardiomyocyte Cells

**Table 1:** Expression of various markers of human cardiac cells at various stages of differentiation.

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<thead>
<tr>
<th>Passage</th>
<th>Gene Expression</th>
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<tr>
<td>1</td>
<td>Kit, Nkx2.5, GATA4, MEF2C, SERCA2a, MHC and Tropinin I</td>
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<tr>
<td>2</td>
<td>Kit, Nkx2.5, GATA4, MEF2C, SERCA2a, MHC and Tropinin I</td>
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<td>Kit, Nkx2.5, GATA4, MEF2C, SERCA2a, MHC and Tropinin I</td>
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<tr>
<td>6</td>
<td>Kit, Nkx2.5, GATA4, MEF2C, SERCA2a, MHC and Tropinin I</td>
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Conclusions:

From this study, we have demonstrated the possibility of ex vivo expansion and differentiation of Human Atrium Cardiac Stem Cells from biopsy specimens in vitro into Functional Human Cardiomyocytes. This provides the possibility of ex-vivo differentiation and expansion of Human Cardiac Stem Cells into functional Cardiomyocytes and subsequent transplantation into patients with pathological impaired or damaged Cardiomyocytes as a result of heart failure. The source of Atrium derived Cardiac Stem Cells Differentiating into functional Cardiomyocytes may provide an alternative source for heart tissue for cardiac transplant patients. Further pre-clinical studies need to be performed prior to introducing a successful Atrium Cardiac Stem cell based therapy for cardiac patients.

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