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

Mariam Majdoub, Natalie Liles, Nina Taw, Gabriel Velazquez, Cristian Sharma, Michael Sharma, Christina DiMaggio, Shaleekha K. Sharma, Sunjay Kaushal, Kalpana Vijayan, Rubio R. Punzalan & Jay P. Sharma

Request for off-prints should be addressed to Jay P. Sharma; Email:stemCells@celprogen.com

CELPROGEN®
Stem Cell Research and Therapeutics

Celprogen Inc.
1871 N. Gaffey St, Ste. A & B, San Pedro, CA 90731
www.celprogen.com

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 P-glycoprotein) properties). The adult Human heart contains small populations of indigenous committed Cardiac Stem Cells (CSC) 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 vitro. 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. Recently, we have successfully isolated Cardiac Stem Cells from small biopsies of Human Myocardium and expanded them ex vivo by many folds without losing differentiation potential into Cardiomyocytes and vascular Cells, bringing autologous transplantation of CSCs 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 disease 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 2-8°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 100% 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% CO_2 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 calcium profile.

Results:

The results are presented in figures 1-19 and graph 1. Briefly, figure 1 indicates the myogenic gene expressions of the following transcriptional factors: cKit, NKx2.5,GATA-4,MEF2C,SERCA2a,αMHC and troponin I of Human Atrium Cardiac Stem Cells.

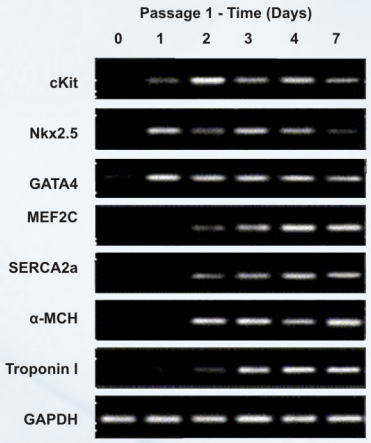
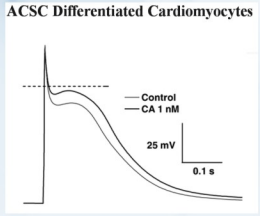


Fig.1 The Expression profile of cardiac-specific markers at the following days 0, 1, 2, 3, 4, 7 at Passage 1..

•RT-PCR analysis of cKit, Nkx2.5, GATA-4, MEF2C, SERCA2a, α-MHC and Troponin-I in P1 Day 1 thru 7 of Atrial Cardiac Stem Cells (ACSC) GAPDH was used as an internal control. *n* = 3.



Graph.1- Atrial Cardiac Stem Cells Differentiated in to Cardiomyocytes Calcium ion channel action potential.

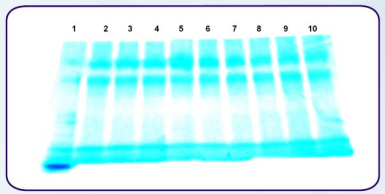


Fig2 - Protein Profile of Human Cardiomyocytes Differentiated from Human Atrial Cardiac Stem Cells.
Lane 1 Molecular weight Marker.
Lane 2-10 are individual Atrial Cardiac Stem Cells Differentiated into Cardiomyocytes.
The protein Gel is a 10% gradient Gel stain with instant blue provided by Expedeon.

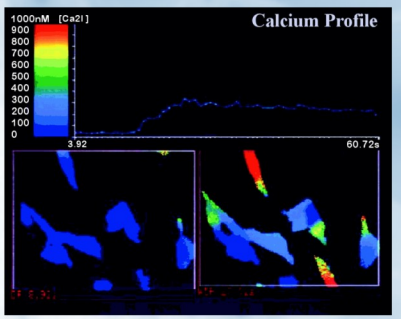


Fig.3 Calcium profile of Differentiated Cardiomyocytes from Human Atrial Cardiac Stem Cells

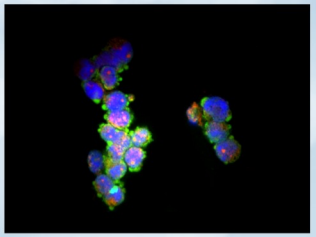


Fig.19 - Cardiac Stem Cell Troponin I with Mitochondria Stain Blue - Nucleus, Green-Troponin-I, Orange Mitochondria.

Conclusions:

From this study, we have demonstrated the possibility of extraction, 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 Atrium Cardiac Stem Cells into functional Cardiomyocytes and subsequent transplantation into patients with physiological 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.

Acknowledgement:

The authors would like to thank Karl J. Kramberger for his technical support and assistance in real time imaging
Abstract Number 2153, B621 - American Society for Cell Biology 48th Annual Meeting - December 13-17 2008 San Francisco, CA

Inmunocytochemistry Characterization of Human Atrial Cardiac Stem Cells

Figures 4-12 Specific Stain for Human Atrial Cardiac Stem Cells (HACSC).

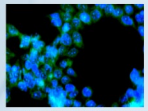


Fig.4 -HACSC SSEA3+ Stain

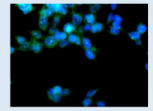


Fig.5 -HACSC SSEA4+ Stain

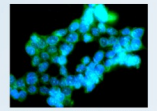


Fig.6 -HACSC TRA1-60+ Stain

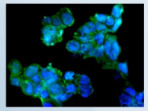


Fig.7 -HACSC TRA1-81+ Stain

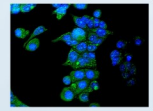


Fig.8 -HACSC cKit+ Stain

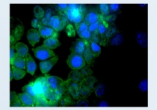


Fig.9 -HACSC KDR+ Stain

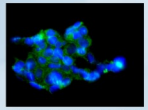


Fig.10 -HACSCNKX2.5+ Stain

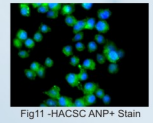


Fig11 -HACSC ANP+ Stain

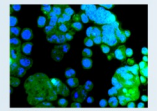


Fig.12 -HACSC SERC2a+ Stain

Inmunocytochemistry Characterization of Human Cardiac Cells Differentiated from Human Atrial Cardiac Stem Cells

Figures 13-18 - Specific Stain for Human Cardiomyocytes Differentiated form Human Atrial Cardiac Stem Cells.

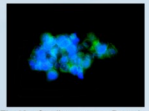


Fig.13 - Cardiomyocytes Desmin+

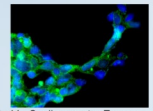


Fig.14 - Cardiomyocytes Tropomyosin+

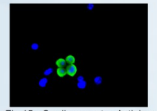


Fig.15 - Cardiomyocytes Actinin+

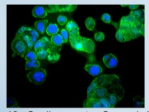


Fig.16 - Cardiomyocytes Connexin43+

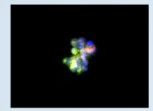


Fig.17 - Human Cardiomyocytes Stained with Tropomiosyn Green, Nucleus Blue, Orange Mitochondria

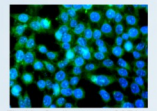


Fig.18 - Cardiomyocyte SSEA1+