"Synchronized Contracting Cardiomyocytes in culture from Human Cord Blood-Derived Stem Cells as a Potential source for Cardiomyocyte-Based Therapeutics and Alternative to Heart Transplantation"

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Introduction:

Ischemic Myocardial damage is one of the increasing causes of heart failure in the western world and is considered irreversible since adult cardiomyocytes are terminally differentiated and for long time have been considered non-proliferating. The reversal of heart failure would require replacement of damaged cardiomyocytes and restoration of blood flow. Celprogen Inc. has focused on Human Cord Blood Stem Cells (HCBSC) to repair damaged myocardial tissue since HCBSC's have been shown to have the plasticity to differentiate in vitro into cardiomyocytes and other cells. The HCBSC's are undifferentiated cells capable of self renewal, proliferation, expansion and differentiation into multiple tissue lineages permitting tissue regeneration. Celprogen has developed its proprietary Cardiomyocyte Differentiation Media that allows HCSBC's to differentiate into synchronized contracting cardiomyocytes in cell culture and also has the ability to expand these differentiated cardiomyocytes in culture. Celprogen's stem cell isolation and expansion technologies allows one to maintain the stem cells in an un-differentiated state and also to expand the un-differentiated stem cell population on a chemically defined extra-cellular matrix without the utilization of mouse feeder layers.

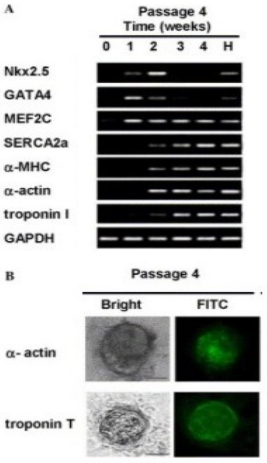
Method:

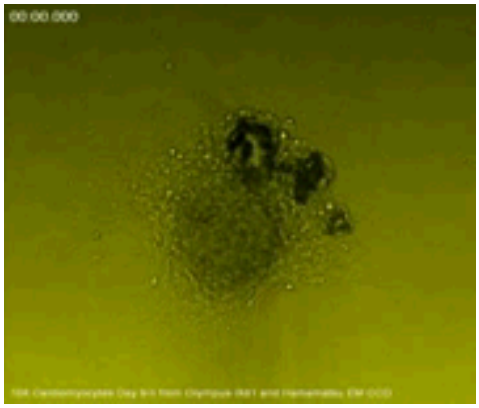
The stem cells were collected from five different human subjects cord blood after normal term delivery, and the stem cells were processed from cord blood with respect to pre-selected expression of nestin positive and CD34 positive stem cells. Human stem cells were extracted from human Cord Blood and seeded onto a culture flask pre-coated with an extra-cellular matrix to maintain the stem cells in an un-differentiated state until passage 2. After passage 2, the cells are transferred to a pre-coated T75 tissue culture flask with cardiomyocyte differentiation matrix and maintained in the Cardiomyocyte Differentiation Media. The expression of myogenic genes was determined by RT-PCR, immunocytochemistry of cardiac muscle markers and real time imaging for calcium ratio studies utilizing Olympus confocal microscope and camera.

Results:

The results are presented in figures 1 through 3 below. Briefly, figure 1 indicates the myogenic gene expressions of the following transcriptional factors: Nkx2.5, GATA-4, MEF2C, SERCA2a, α-MHC, α-sarcomeric actin (α-actin), and troponin I at passage 4 of cardiomyocytes differentiated from stem cells. The images in B of figure 1, shows the presence of immunological markers α-actin and troponin I as positive markers for

cardiomyocytes. The image in C of figure 1 shows the contracting cardiomyoctes still photograph at day 7 of passage 2 in cell culture.





C: Passage 2, day 7 of differentiation in Culture

Figure 1: Stem cells transdifferentiated into cardiomyocytes. The Expression profile of cardiac-specific markers at the following passages P4. (A) RT-PCR analysis of early cardiac transcriptional factors Nkx2.5, GATA-4, MEF2C, SERCA2a, α-MHC, α-sarcomeric actin (α-actin), and troponin I in P4 of transdifferentiated cardiomyocyte at 0, 1, 2, 3, and 4 weeks after in Celprogen's Cardiac myocyte differentiation Media. H, positive control from the rat neonatal heart. GAPDH was used as an internal control. *n* = 5. (B) Representative immunocytochemistry for cardiac α-sarcomeric actin (α-actin, upper panel) and troponin T (lower panel) in P4, of transdifferentiated cardiomyocytes at 4 weeks after cultured in Celprogen's Cardiac Differentiation Media and on Celprogen's Cardiac Myocyte extra-cellular matrix. *n* = 5. Scale bar = 100 µm

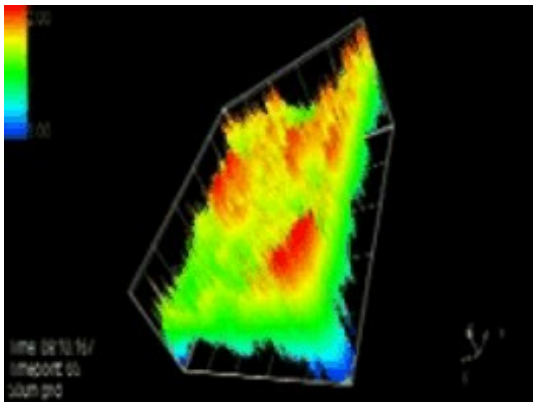


Figure 2: Calcium ratio of contracting cardiomyocytes at P4 at day 14 imaged with Calcium imaging dye Calcium Green -5N, AM obtained from Molecular Probe.

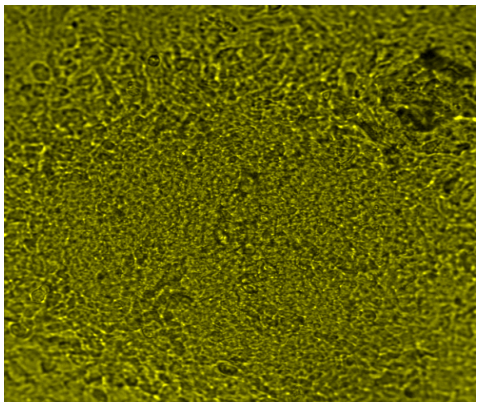


Figure 3: Transdifferentiated Cardiomyocytes Passage 1 and day 7 in culture.

The figure 2 indicates calcium ratio of contracting cardiomyocytes at day 14 of passage 4, the red regions of the graph indicates high calcium whereas blue regions on graph indicate low calcium with green assigned to medium on a scale of 0-2.0.

Conclusion:

From this study, we have demonstrated the possibility of pre-selecting and differentiating human umbilical cord blood stem cells *in vitro* into cardiomyocytes capable of synchronized contraction. This provides the possibility of ex-vivo differentiation and

expansion of cord blood stem cells into contracting / functional cardiomyocytes and subsequent transplantation into patients with physiological impaired heart or damaged cardiomyocytes as a result of heart failure. The source of cord blood derived stem cells trans-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 stem cell based therapy for cardiac patients.

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