Human Fibroblast / Keratinocyte De-differentiation into Human Cardiomyocytes in Tissue culture

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Somatic Cell De-differentiation provides an emerging strategy to produce embryo-independent Pluripotent Stem Cells from somatic tissue. Induced Pluripotent Stem Cells (iPC) demonstrate aptitude for de novo Cardiac differentiation, yet their potential for heart disease therapy has not been tested. In this study, Human Fibroblasts / Keratinocytes were transduced utilizing non-viral mechanisms with Human stemness factors OCT3/4, SOX2, and c-MYC converted into an Embryonic Stem Cell-like phenotype and demonstrated by their ability to spontaneously assimilate into preimplantation host morula via diploid aggregation, unique to pluripotent cells. The iPCs were differentiated into normal heart tissue. The genetic and protein profiles of the differentiated cardiac patterning were comparable to normal Human Cardiac tissue. Human Fibroblasts / Keratinocytes De-differentiation with Human Stemness factors may provide an alternative potential to repair acute Myocardial infarction, or in the understanding of novel drug discovery potential for heart disease.

Methods: Skin biopsy specimens were taken from five individuals and primary Fibroblast / Keratinocyte cell cultures were establish utilizing Celprogen Keratinocyte Complete Growth Medium in pre-coated Keratinocyte Extra-cellular Matrix (ECM) T25 flasks. At passage 2 the primary cell cultures were trypsinized and transferred to the human iPCs De-differentiation Media and iPCs De-differentiation pre-coated T25 Flask with Matrix. Upon generation of the Embryoid Bodies the cultures were maintained in Embryoid Body Media and Matrix for 7 days in culture. At the end of the 7th day the Embryoid Bodies were transferred to Cardiomyocyte Differentiation Media and Matrix and cultured in this Media and Matrix for 14 days. At the end of 14 days in Human Cardiomyocyte Differentiation Media and ECM the cells were profiled for cardiac gene expression and protein profile signatures for cardiomyocytes.

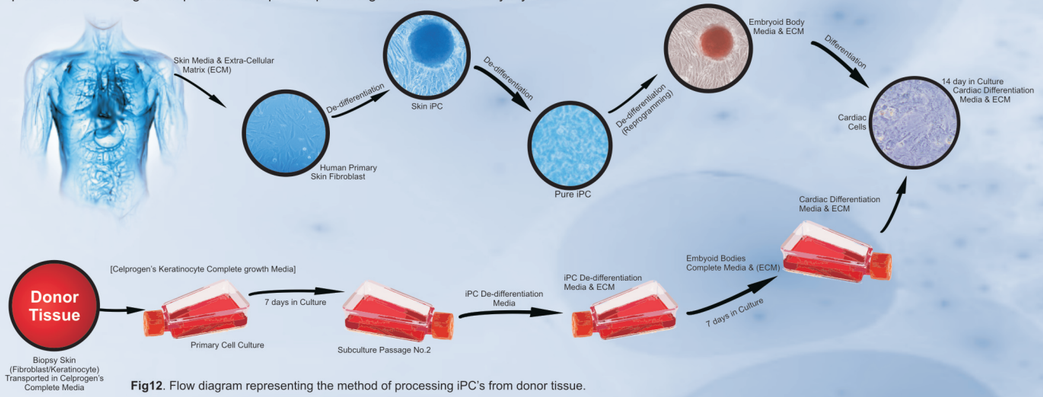


Fig12. Flow diagram representing the method of processing iPC's from donor tissue.

Results: The results are indicated in Graph 1 and Figures1-12.

Conclusions: From the present study we can demonstrate that adult somatic Cells (Fibroblast / Keratinocytes) were De-differentiated (reprogrammed) into their progenitor cell stage and then further de-differentiated into their resident pool of embryonic stem cell like linage. The de-differentiated somatic Cells were further differentiated to cardiomyocytes with cardiomyocyte molecular and protein signatures unique to cardiomyocyte phenotype. We need to perform long term studies in-vivo system prior to making this non viral method feasible for clinical setting

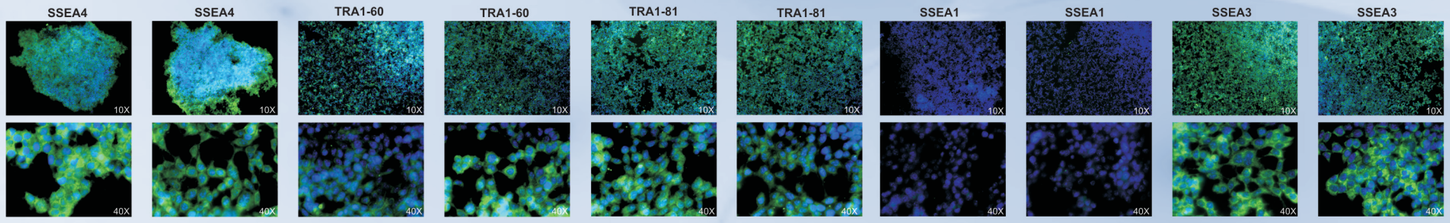


Fig10. iPC Characterization with the following markers: SSEA4, TRA1-60, TRA1-81, SSEA1and SSEA3. Utilizing inmunohistochemistry (IHC).

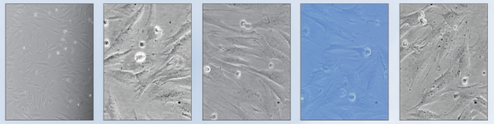


Fig1. Human Donor Skin / Keratinocyte Cells prior to being induced for iPC generation

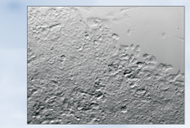


Fig2. Mixed iPC Culture with Non-iPC Cells

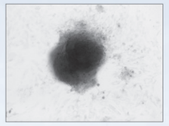


Fig3. Mixed iPC Culture (Cluster: iPC)

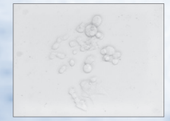


Fig4. Human iPC Generated from Skin Selected iPC Clones

Fig5. Selected iPC Clones for Expansion

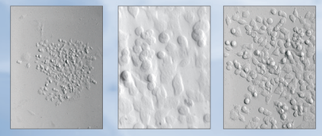


Fig6. Cloned iPC Cells - Sox2, Oct3/4, Klf4, cMyc (Expansion Media and Matrix)

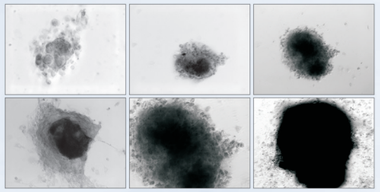


Fig7. Differentiated iPC Embryoid Bodies in Celprogen's Human Embryoid Body Media and Matrix

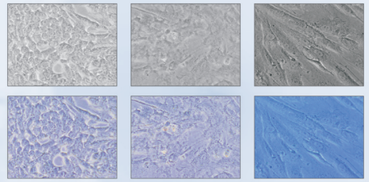


Fig8. Differentiated iPC to Embryoid Bodies to Cardiomyocytes in Celprogen's Human Cardiomyocyte Differentiation Media and ECM.

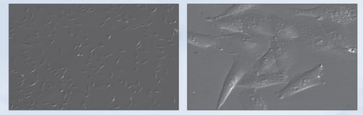
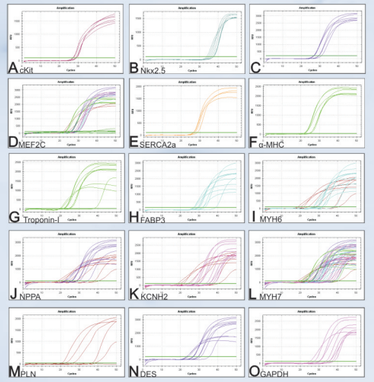


Fig.9 Fully differentiated Human Cardiomyocytes from iPC's



Graph1. Realtime PCR data from the following Cardiac genes: A. cKit, B. Nkx2.5, C. GATA-4, D. MEF2C, E. SERCA2a, F. α-MHC, G. Troponin-I, H. FABP3, I. MYH6, J. NPPA, K. KCNH2, L. MYH7, M. PLN, N. DES, AND O. (ACSC) GAPDH.
(Real Time - PCR: CFX96™ Real-Time System C1000™ Thermal Cycler)

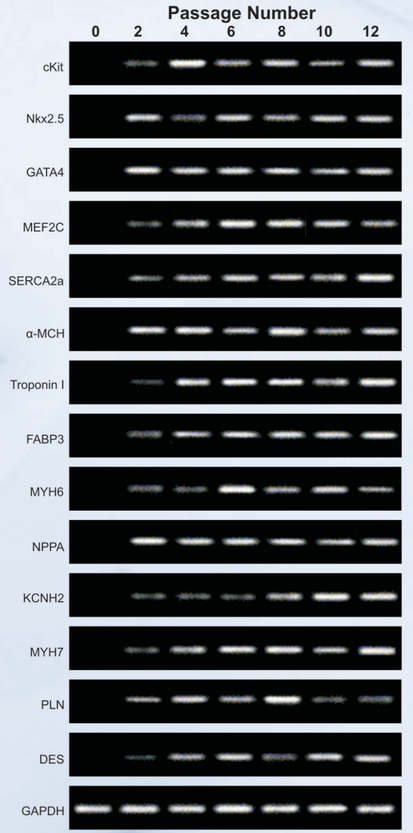


Fig.11 The Expression profile of cardiac-specific markers at the following Passages 0, 2, 4, 8, 10, 12. RT-PCR analysis of cKit, Nkx2.5, GATA-4, MEF2C, SERCA2a, α-MHC, Troponin-I, FABP3, MYH6, NPPA, KCNH2, MYH7, PLN, and DES of Atrial Cardiac Stem Cells (ACSC) GAPDH was used as an internal control. *n* = 5.

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