Conversion of human adipogenic mesenchymal stem cells into highly conductive myocytes

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ABSTRACT. In a unique manner human transcription factors ETS2 and MESP1 were sufficient to convert human adipogenic mesenchymal stem cells (hAdMSC) into cardiac progenitor cells (CPCs). These two factors up regulate a cadre of cardiac regulatory factors, Nkx2.5, Tbx5, Mef2C, dHAND and GATA4. Yet, they are unable to produce the appearance of mature myosin heavy chains and many calcium-handling proteins. Nevertheless, the addition of epinephrine was capable of promoting maturation of the electrophysiological and Ca2+ handling properties of hAdMSC converted CPCs. Adrenergic signaling through *Beta Adrenergic 2 receptor* repositioned converted CPCs into more mature myocytes cells, along with the appearance of *RYR2*, *CAV2.1*, *CAV3.1*, *Nav1.5*, *SERCA2* and *CX45* gene transcripts. Following treatment with epinephrine, action potentials were observed in (Nkx2.5-puromycin) drug selected myocytes. Further improvement was fostered by 3D cardio-spheroids formed in a Synthecon, Inc. rotating bioreactor (RCCS). These cardio-spheroids induced the appearance of hypoxic genes: *HIF1a/b*, *PCG1*a/b and *NOS2*. Induction of the hypoxic program coincided with the robust activation of adult contractile genes, *MYH6*, *MYH7 and TNNI3*, ion channel genes, *CACNA1C, SCN8a, KCNQ5, KCNQ3* and t-tubule genes, *CASQ, JPH, ASPH PLN, TRDN, BIN1 and CALR*. These myocytes were found to be electrically coupled and conduct at high rates. Additional in-depth studies demonstrated the appearance of hyperpolarization-activated and cyclic nucleotide-gated channels (*HCN1-4*). Our experimental paradigm contributes to novel regenerative strategies that enhanced maturation of converted hAdMSC's to electrical active myocytes.

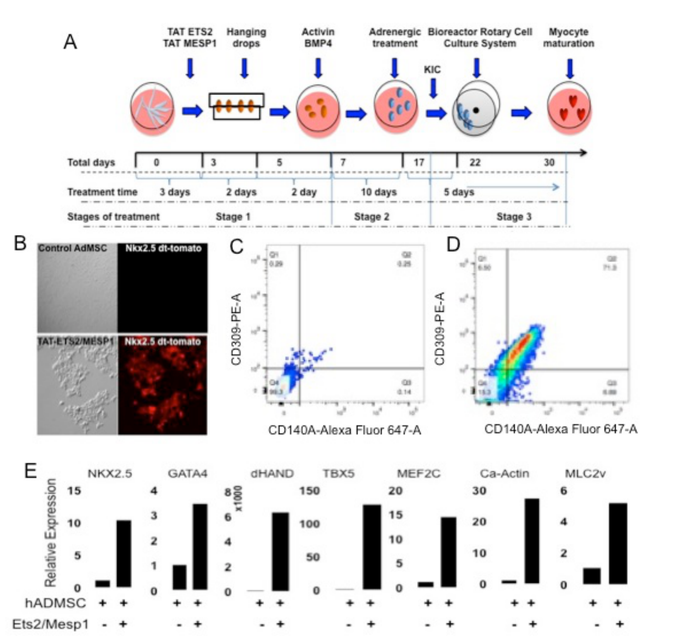

Figure 1. A three stage protocol for converting hAdMSCs into cardiac progenitors and highly conductive myocytes. A. Schematic diagram shows 3 stages of reprogramming and maturation. **Stage 1** constitutes protocol used to reprogram human fibroblasts using TAT-ETS2 and TAT-MESP1 proteins. **Stage 2** β-adrenergic stimulation with epinephrine to enhance cardiac myocyte Ca_2^+ management. **Stage 3** Use Synthecon RCCS bioreactor to form 3D cardio-spheres. B. Cell morphology shown by the appearance of NKX2.5 red td-Tomato reporter. C,D. FACS analysis for cardiac markers KDR/VEGFR (CD309) and PDGFRa (CD140a) and E. Appearance of Nkx2.5, and GATA4, HAND2 and TBX5 contractile proteins genes, MLC2V, and cardiac α-actin.

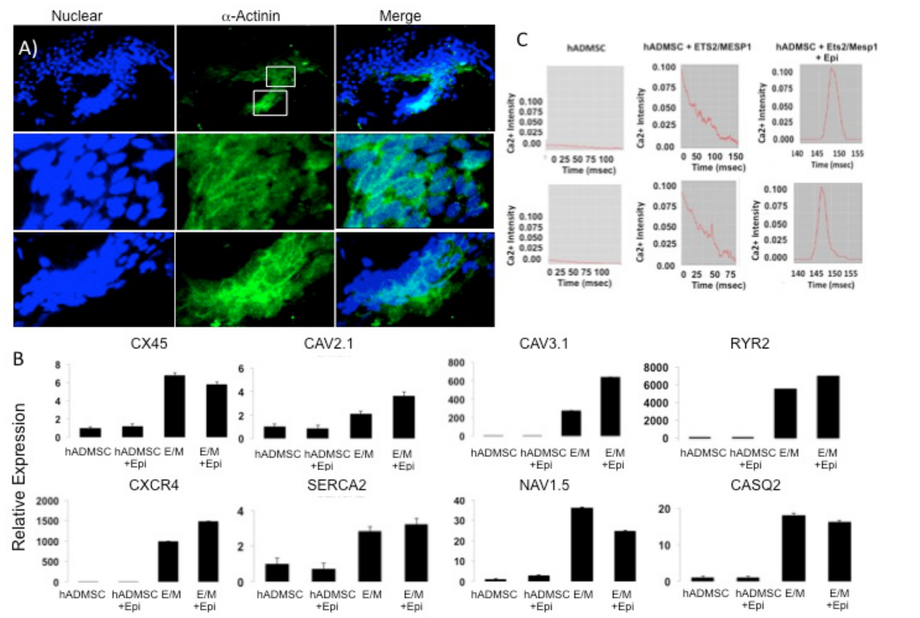

Figure 2. Myofibrilogenesis with the appearance of thin filament I-Z-I staining (anti a-actinin antibody) and expression of *RYR2*, L- and T-Type Ca2+ channels: *CAV1.2* and *CAV3.1*. A. Shows cell morphology for Stage 2. (Dapi/α-Actinin). B. Shows upregulation of core Ca^{2+} management genes. C. Calcium analysis using IC200, shows calcium fluctuation during differentiation.

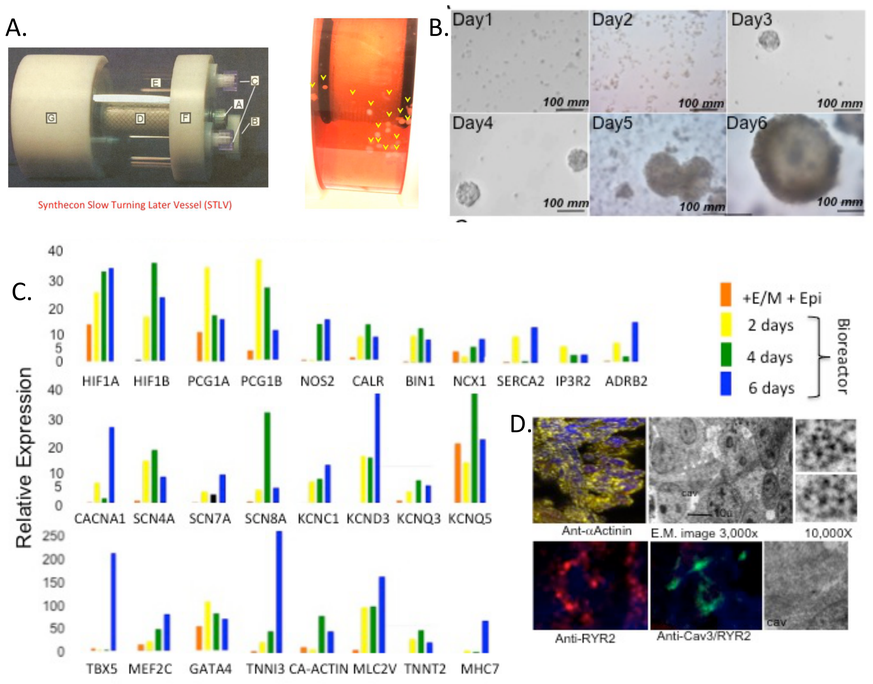

Figure 3. **Converted hAdMSCs form 3D cardio-spheres in the Synthecon RCCS.**A. Left. Slow turning lateral vessal RCCS reactor (Syntecon ®), right, ETS2/MESP1 stage 3 cells . B. Cell growth over 6 days. C. . RNA expression analysis showed up-regulation of a hypoxic program. Contractile proteins including adult myosin heavy chain genes *MYH7* and thin filament proteins cardiac a-actin, MLC2v and TNNT2 and ion channel genes, CACNA1, SCN4A, SCN7A, SCN8A, KCNC1, KCND3, KCNQ3, KCNQ5, and SR and t-tubule genes, CASQ, JPH, ASPH, PLN, TRDN, BIN1, CALR, NCX1 and CASQ2. D SR and T-tubule markers correlated with appearance of immuno-stained RYR2 adjacent to nascent caveloae stained with anti-Cav3 antibody. Electron microscopic images also displayed caveloae vesicles that will coalesce to form t-tubules.

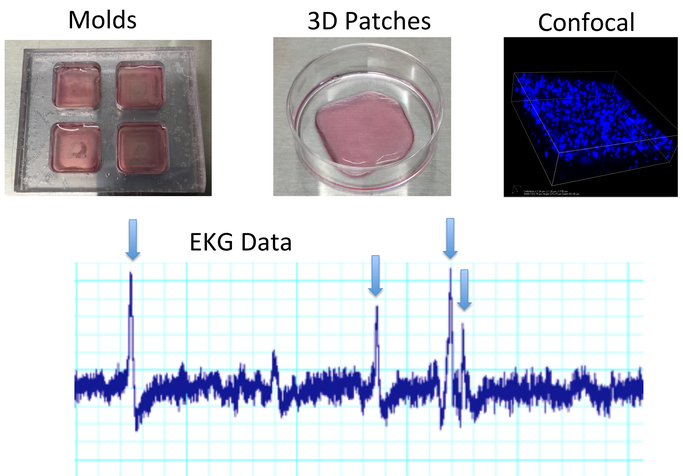

Figure 4. **Electrocardiogram of highly conductive cardiomyocytes.** Cells were cultured in 3D patches (tissue simulation) and EKG measurement was performed**.** Data indicates continuous electrical activity. Arrows depict **QRS complex** appearance.

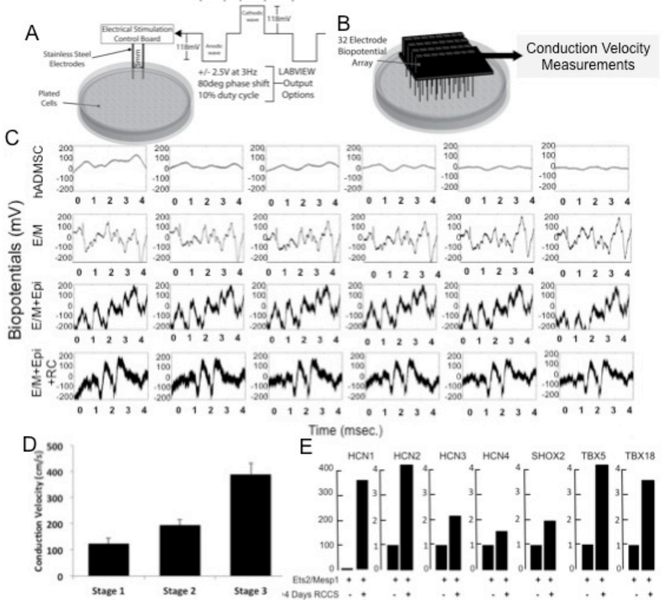

Figure 5. Biopotentials and velocity. A, B. Representation of a 32 electrode system used to measure biopotential and velocity recordings.. Maturation coincides with development of higher amplitude and more rhythmic biopotential peaks. **B.** Bulk conduction velocities, measured from the amplitude lags between rhythmic waveform patterns coincident in multiple electrodes and electrode distances, increased during the three stages of maturation. **C)** Up regulation of key electrical conductivity genes tied to HCN channels coincides with measured improvement in biopotential and increased conduction velocities.

References:

Islas, J. F. *et al.* Transcription factors ETS2 and MESP1 transdifferentiate human dermal fibroblasts into cardiac progenitors. *Proc. Natl. Acad. Sci.* **109,** 13016–13021 (2012).

Liu, Y. & Schwartz, R. J. A driver of cardiac cell fate determination *Transcription*.**4,** 92–96 (2013)

Michel, M. C., Harding, S. E. & Bond, R. a. Are there functional β_3-adrenoceptors in the human heart? *Br. J. Pharmacol.* **162,** 817–822 (2011).

Cerignoli, F. *et al.* High throughput measurement of Ca^{2+} dynamics for drug risk assessment in human stem cell-derived cardiomyocytes by kinetic image cytometry. *J. Pharmacol. Toxicol. Methods* **66,** 246–56 (2012).

Bers, D. M. Adrenergic Fight-or-Flight: S-NO Falls on PKA Targets. *Circ. Res.* Genes Dev. **117,** 747–9 (2015).

Birla, R. K., Borschel, G. H., Dennis, R. G. & Brown, D. L. Myocardial engineering in vivo: formation and characterization of contractile, vascularized three-dimensional cardiac tissue. *Tissue Eng.* **11,** 803–13

Salazar, B., Reddy, A., Tao, Z., Madala, S. & Birla, R. 32-Channel System to Measure the Electrophysiological Properties of Bioengineered Cardiac Muscle. *IEEE Trans. Biomed. Eng.* (2015).

Synthecon. *The Rotary Cell Culture SystemTM RCCS-1/RCCS-4 The Premier Tissue Engineering Culture System. User Guide*