Heterogeneity of Myocardial ATP Flux Rate via CK In Vivo Porcine Hearts with hiPSC Tri-lineage Cell Transplantation Using 2D CSI P-31 MR Spectroscopy

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Purpose: hiPS cells hold promise for myocardial repair, but preclinical studies are needed in large animal models to determine the optimal delivery strategy, best cell preparation, and safety. We investigated the functional impact and myocardial heterogeneity of myocardial bioenergetics using combined intramyocardial transplantation of cardiomyocytes, endothelial cells, and smooth muscle cells derived from hiPSCs to a porcine model of ischemia reperfusion (I/R) , and 2D CSI P-31 MR spectroscopy.

Methods: Ischemia-reperfusion injury was surgically induced in female Yorkshire farm swine (≈15kg), then randomly assigned to 2 experimental groups: 1) 6 million Human induced pluripotent stem cells (hiPS cell) derived cardio myocytes (CMs)), smooth muscle cells (SMC) and Endothelia cells (ECs) were directly myocardial injected, 2) open patch (fibrin patch with no cell) were placed over the injury site. Four weeks later, MR studies were performed using a 9.4 T Varian (65cm bore) system. A home built 28mm $^{1}H/^{31}p$ surface coil was sutured directly to the left ventricle epicardium over the peri-infarct region. ^{31}P MR spectrums were acquired with adiabatic half passage RF pulses for excitation to minimize flip angle variation due to B1 inhomogeneity from the surface coil [1]. To measure the myocardial flux of the PCr→ATP and ATP→Pi, selective saturation of ATPγ or of both PCr and Pi was achieved by using the B1-insensitve train to obliterate signal technique. PCr/ATP ratio, $K_{pcr\rightarrow ATP}$ (the creatine kinase reaction rate constant) and $K_{ATP\rightarrow Pi}$ (the ATP hydrolysis reaction rate constant) were calculated according to previously reported method [2,3].

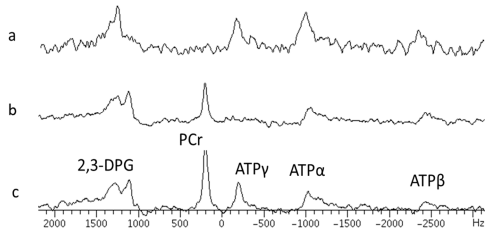

Fig1. Magnetization saturation transfer experiment to measure pig myocardial ATP turnover rate (TR=6.8s, NEX=16). a) spectrum with both PCr and Pi saturation($K_{ATP\rightarrow Pi}$ =0.13s^{-1}). b) spectrum with γ-ATP saturation ($K_{pcr\rightarrow ATP}$ =0.32s^{-1}). c) baseline spectrum without saturation (PCr/ATP=1.92)

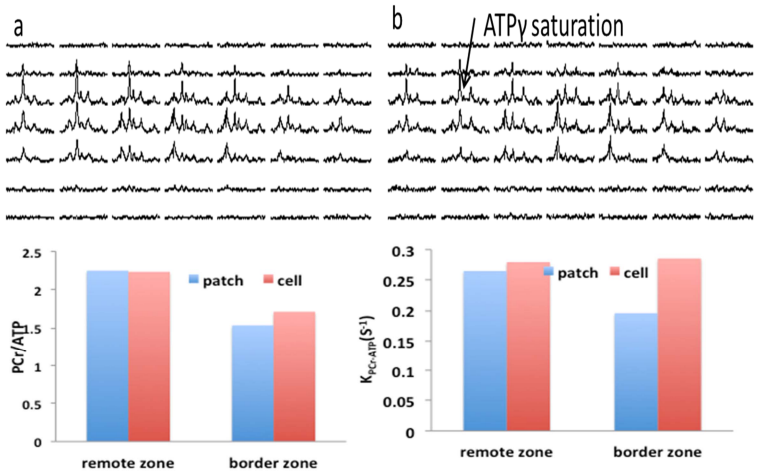

Fig2. In vivo myocardial energetic mapping was achieved with ^{31}P CSI2D (TR=2.7s, NEX=12, FOV=40mmx60mm, 8x10 phase encodes, Thk=40mm). Figure a and b showed the ^{31}P CSI2D stacked spectrum of pig left ventricle without saturation (a) and with the γ-ATP saturation; c and d , summarized data of myocardial PCr/ATP and $K_{pcr\rightarrow ATP}$ for two groups. There is no significant difference observed for both PCr/ATP ratio and $K_{pcr\rightarrow ATP}$ on remote zone. PCr/ATP ratio is significantly increased ($p<0.05$) in ips cell treated group compared with open patch group on border zone.

Results: Fig1 demonstrated that ATP turnover rate could be measured with two components, the creatine reaction rate and ATP hydrolysis rate, even without measuring Pi levels. The data in fig 2 showed that MI injury has a heterogeneous effect on myocardial bioenergetics. PCr/ATP ratio (remote vs border =2.25 vs 1.54) is significantly decreased in border region of the infarction in both groups, and the border zone PCr/ATP ratio is improved in the cell treated group compared with open patch group. The $K_{pcr\rightarrow ATP}$ in the border zone is slightly increased in cell treated group compared with patch only group.

Discussion and Conclusion: The post mortem immunostaining showed that ECs and SMCs were detected in vascular structures, and hiPSC-CMs were found integrated into the myocardium and exhibited organized sarcomeric structure four weeks after the cell applied to the epicardial surface of I/R myocardium, which is consistent with the findings of improvements of ATP flux rate via CK. This protocol established the proof of concept for safety and efficacy of Tri-lineage cell transplantation using a clinically relevant porcine model of post infarction LV remodeling.

Reference**:** **1**. Q xiong, F Du, X Zhu, P zhang, P Suntharalingam, J Ippolito, F.D. Kamdar, W Chen, J Zhang, ATP Production Rate via Creatine Kinase or ATP Synthase In Vivo: A Novel Superfast Magnetization Saturation Transfer Method, Circulation Research, 2011; 108: 653-663 **2**. Q xiong, L Ye, P zhang, Mi Lepley, L zhang, C Swingen, J Vaughan, D.S. Kaufman, J Zhang, Bioenergetic and Functional Consequences of Cellular Therapy: Activation of Endogenous Cardiovascular Progenitor Cells, Circulation Research, 2012; 111:455-468. **3**. Q xiong, L Ye, P zhang, Mi Lepley, J Tian, J Li, L zhang, C Swingen, J Vaughan, D.S. Kaufman, J Zhang, Functional Consequences of Human Induced Pluripotent Stem Cell Therapy: Myocardial ATP Turnover Rate in the In Vivo Swine Heart With Postinfarction Remodeling; Circulation, 2013; 127:997-1008. ***Acknowledgements*****:** HL95077, HL114120, NIH P41 EB015894, WM KECK Foundation