Manganese guided cellular MRI of human embryonic stem cell viability

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Introduction: Human embryonic stem cells (hESC) have demonstrated the ability to restore the injured myocardium. MRI has emerged as one of the predominant imaging modalities using iron-oxide nanoparticles to localize the transplanted stem cells. However, this method does not monitor biological activities of the transplanted cells including cellular viability. Manganese chloride ($MnCl_2$) is transported via calcium-channel into the cellular cytoplasm to shorten cellular T1 and generate positive contrast indicative of cellular viability. We tested the hypothesis that $MnCl_2$ will enable *in vitro* MRI assessment of hESC viability.

Method: **hESC culture:** hESC (H9, Wicell, Madison, WI) stably transfected with luciferase reporter gene (hESC-luc$^+$) was cultured on mouse embryonic fibroblast feeder layer or on growth factor reduced matrigel with hESC growth media consisting of basic fibroblast growth factor. The hESC-derived cardiomyocytes (hESC-CM, contractile cells) were generated from embryoid bodies (EBs) and enriched by Percoll gradient. **Mn Labeling:** We labeled about 0.5 - 3x10^6 trypsinized hESC with 0.01-3.00 mM of $MnCl_2$ in normal saline and incubated for 0.5-1.0 hour at 37°C. Biological properties of hESC were monitored by modulating the activity of calcium channels using verapamil (calcium channel antagonist) and (S) (-) Bay K 8644 (calcium channel agonist). **MRI:** T_1 and T_2 mapping was performed at 0.01-3.00 mM of $MnCl_2$ solution with 1.5 T GE Excite whole-body MRI scanner (Signa, GE Medical Systems, Milwaukee, WI) with a 5-inch receive only surface coil. For T1 measurements, spin echo (SE) inversion recovery sequence (FOV 12 cm, matrix size of 128x128, TR 3000 ms and TE 50-2200 ms at 300 ms steps) were used. We made T2 measurements using SE sequence (FOV 12 cm, matrix size of 128x128, TR 2500 ms and TE 10-80 ms at 10 ms steps). Then the data were analyzed to extract T1 and T2 values through nonlinear least-square fits to the SE inversion recovery and the SE decay curve respectively. *In vitro* cellular MRI was performed using optimized SE sequence (FOV 12 cm, matrix size of 256x256, TR 800 ms and TE 3.4 ms). Modulation of hESC calcium channel activity by verapamil and (S) (-) Bay K 8644 was assessed by measuring changes in signal intensity. The $MnCl_2$ signal of hESC-luc$^+$ viability was validated by bioluminescence imaging (BLI, Xenogen IVIS System 200, CA). Metabolic effects of $MnCl_2$ on hESC were tested using MTT assay for cellular proliferation and flow cytometry detection of Annexin V for apoptosis.

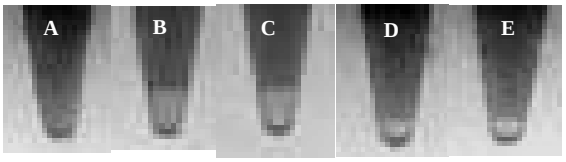


Figure 1: MRI of hESC and hEB in different Mn concentration (A) control (hESCs in normal saline), (B) 0.10 mM Mn, (C) 0.10mM with 5 μM Dobutamin, (D) 0.10 mM Mn, (E) 0.10mM with 250 μM Verapamil

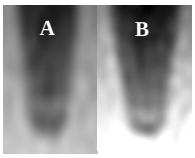


Figure 2 (A) undifferentiated hESC (uhESC) in 0.10 mM Mn (B) contractile hESC-CM in 0.10 mM Mn

Figure 3

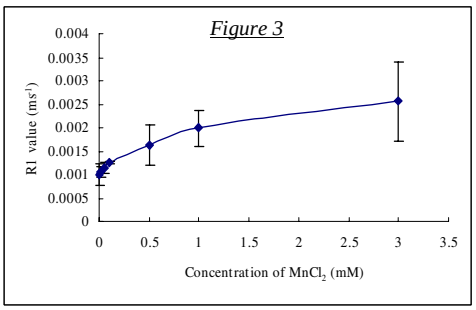
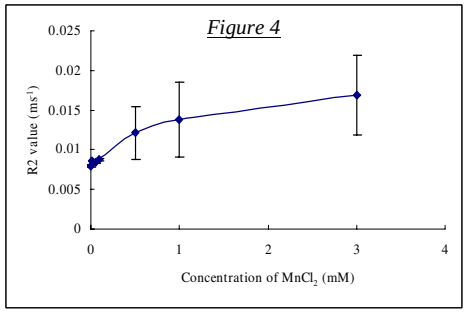


Figure 4



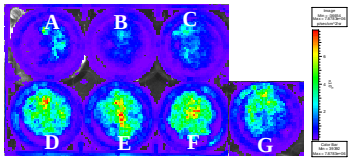


Figure 5: BLI images of hESC in different concentration of Mn (A) control (hESC in normal saline), (B) 0.01 mM, (C) 0.05 mM, (D) 0.10 mM, (E) 0.5 mM, (F) 1.0 mM, and (G) 3.0 mM

Results: *In vitro* cellular MRI indicates that viable hESC take up increasing amount of $MnCl_2$ with increasing extracellular concentrations of $MnCl_2$ as seen by respective increase in T1-shortening effects (Figure 1). Calcium-channel mediated biological properties of hESC were confirmed by the reduction of difference in signal intensity by 24% when 250 μM verapamil was co-administered with 0.10 mM $MnCl_2$ (p<0.05, 4264±227 versus 4094±248, n=4, Figure 1). A trend towards increased signal intensity was observed when 5μM (S) (-) Bay K 8644 was co-administered with 0.10 mM $MnCl_2$ (915±72.1 versus 1126±30.7, n=2, Figure 1). In addition, signal intensity was measured between undifferentiated hESC and contractile hESC-CM demonstrating increased signal intensity (50%) in hESC-CM (97.2 vs. 147.6, respectively, Figure 2). Furthermore, T1 and T2 relaxation times of Mn labeled hESC have been measured (Figure 3 and 4). Finally, positive BLI signal of hESC validated the MRI findings of cellular viability (Figure 5). MTT and flow cytometry assays demonstrated that $MnCl_2$ labeling did not significantly impact cellular metabolism including cell proliferation and apoptosis.

Discussion: $MnCl_2$-guided cellular MRI demonstrates the potential to detect cellular viability and biological properties including calcium channel activity and contractility of hESC-CM. This technique may enable precise *in vivo* MRI of the biological properties of transplanted hESC.

References: [1] Aoki I, et al. *NMR Biomed* 2006; 19(1):50-59. [2] Bruvold M et al, *Invest Radiol* 2005;40: 117-125.