Manganese guided cellular MRI of human embryonic stem cell viability

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Objective Human embryonic stem cells (hESC) have demonstrated the ability to restore the injured myocardium (1). 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 cellular viability. In order to address the issue, this investigation tests the hypothesis that $MnCl_2$ (manganese chloride) will enable *in vitro* MRI assessment of hESC viability. It has been known that $MnCl_2$ enters the cells via calcium (Ca^{2+}) channels when the cells are biologically active, thus, indicating cellular viability (2).

Methods **hESC culture:** hESC (H9, Wicell, Madison, WI) was stably transfected with luciferase reporter gene (hESC-luc$^+$). hESC-luc$^+$ culture was maintained on mouse embryonic feeder layer and hESC growth media (80% knockout DMEM, 20% serum replacement, 1% Non essential amino acid, 1mM L-glutamine 1 mM/100x, 0.1mM β-mercaptoethanol 3.5 uL to 500 ml total solution with h-bFGF4ng/ml). **Differentiation of hESC:** hESC were dissociated and cultured in cell cluster suspension using low attachment plates and differentiation medium (80% KO-DMEM, 1 mmol/L L-glutamine, 0.1 mmol/L ß-mercaptoethanol, 1% nonessential amino acids stock, and 20% FBS) to form embryoid bodies (EBs). After 4 days in suspension, EBs were transferred to gelatin coated plates at 1 to 3 EBs/cm^2 and cultured for 10-15 days. The hESC-derived cardiomyocytes (hESC-CM, contractile cells) were enriched by applying onto a discontinuous Percoll gradient. **Mn Labeling:** Before labeling with $MnCl_2$, hESC were trypsinized and dissociated into single cells. 0.01, 0.05 and 0.10mM of $MnCl_2$ in normal saline were made prior to the labeling to minimize oxidation. hESC were incubated for 2-3 hours at 37°C. hESC quantity of 0.5 - 3x10^6 were labeled. Cell numbers were counted and their viability was confirmed prior to labeling. **Bioluminescence imaging (BLI) of cell viability:** The viability of hESC-luc$^+$ has been validated by bioluminescence imaging (BLI, Xenogen IVIS System 200, CA). The optical signal generated by molecular expression of luciferase reporter gene confirms the viability of hESC-luc$^+$ (Figure 2). **MRI:** The following pulse sequences using 1.5 T clinical scanner (Signa, GE, Milwaukee, WI) were implemented: Spin Echo (SE) TR=800 ms, TE=10 ms., NEX=1, 256x256, FOV=12; spin echo inversion recovery (SE-IR) TR=500-800 ms, TE=min., TI=500-800 ms, NEX=1, 256x256, FOV=12.

Results MRI signal for $MnCl_2$ concentration of 0.01-0.10 mM using SE sequence demonstrate increased signal intensity (SI) due to the T1-shortening effect by intracellular $MnCl_2$ accumulation (Figure 1). *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 2A-B). The functional property of Ca^{2+} channels of hESC is confirmed by the reduction of SI by 32% (1638±131 vs. 1268±101) when verapamil (Ca^{2+} channel-blocker) is co-administered with 0.1mM $MnCl_2$ (Figure 2B-C). Finally, SI was measured between undifferentiated hESC (uhESC) and contractile hESC-CM. hESC-CM demonstrated 1.2-fold increase of MR signal consistent with increased expression of Ca^{2+} channels in the contractile hESC-CM (2070 vs. 1744) (Figure 2D-E). Finally, positive BLI signal of hESC validates the MRI findings of cellular viability (Figure 3).

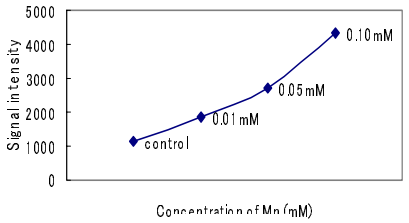

Figure 1

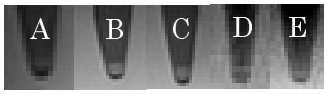

Figure 2

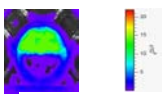

Figure 3

Fig.1: Signal intensity difference with various concentration of Mn. **Fig.2:** MRI of hESC and hEB in different Mn concentration (A) control (hESCs in normal saline), (B) 0.10 mM Mn, (C) 0.10mM with 250 uM Verapamil, (D) undifferentiated hESC (uhESC) in 0.10 mM Mn., and (E) contractile hESC-CM in 0.10 mM Mn. **Fig. 3:** BLI of hESC culture.

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

References

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(2) Bruvold M et al, Invest Radiol 2005;40: 117-125.