In vitro evaluation of cellular engraftment parameters of 3 transfection methods to label mouse embryonic stem cells using ferumoxides

Y. Suzuki[1], C. H. Cunningham[2], M. Drukker[3], P. C. Yang[1]

[1]Cardiovascular Medicine, Stanford University, Stanford, California, United States, [2]Electrical Engineering, Stanford University, Stanford, California, United States, [3]Pathology, Stanford University, Stanford, California, United States

Background Magnetic resonance tracking of stem cells is an emerging application designed to monitor cell engraftment *in vivo*. Much effort has been directed towards developing efficient transfection methods to facilitate intracellular magnetic labeling with SPIO (ferumoxides). Although techniques utilizing poly-L-lysine (PLL), protamine sulfate (PS), and electroporation (Elp) have been proposed, the effects of these 3 transfection methods on cell engraftment parameters have not been investigated systematically.

Objectives *In vitro* comparison of the engraftment parameters of magnetically labeled mESC with the 3 transfection methods using ferumoxides as a contrast agent was conducted. Critical biological determinants of cellular engraftment including longitudinal viability, differentiation capacity of mESC into cardiomyocyte, and iron content of mouse embryonic stem cells (mESC) were compared among the 3 transfection techniques.

Materials and Methods Mouse ESC line transfected with Click Beetle Red luciferase reporter gene (mESC-luc$^+$) and cardiomyocyte lineage specific promoter Nkx2.5 linked to downstream Green Fluorescent Protein marker (mESC-Nkx2.5-GFP$^+$) were labeled using the PLL, PS, and Elp protocols as previously described [1-3]. For assessment of cellular viability and proliferation of labeled mESC-luc$^+$, a 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay and bioluminescence imaging of CBR luciferase gene activity were conducted. Differentiation capability of the magnetically labeled mESC-Nkx2.5-GFP$^+$ was assessed using differentiation protocol consisting of 150ng/ml noggin (R&D Systems, Minneapolis, MN) stimulation, an early cardiomyocyte differentiation agonist[4]. At day 10 following the formation of embryoid bodies (EB) from undifferentiated mESC and induction of cardiomyocyte differentiation, the percentage of differentiated EB defined as spontaneously contractile EB under microscopic observation was measured and the percentage of Nkx2.5-GFP$^+$ cells were analyzed with flow cytometer (BD Sciences, Franklin Lakes, NJ). Finally, iron content of $2x10^6$ mESC was compared by measuring the largest area of MR signal dephasing among the 3 transfection methods.

Results The longitudinal cellular viability and proliferative capability of the 3 transfection methods demonstrated no significant difference (p=NS) among the 3 techniques and also when compared to unlabeled cells as shown by MTT assay (Table 1) and bioluminescence imaging (Figure 1) at days 1, 4, 7, and 10. The differentiation capacity of mESC into cardiomyocytes also demonstrated no significant difference among the 2 transfection groups (PLL and PS) and unlabeled mESC as determined by % of beating embryoid bodies and % of Nkx2.5-GFP$^+$ cells (shown in parentheses) while the Elp group demonstrated significantly lower differentiation capacity: unlabeled control 83.3% (1.73%), PLL 66.7% (1.39%), PS 83.3% (1.53%), Elp* 58.3% (0.62%) (*p<0.05). Finally, the PS group generated the largest area of dephasing signal consistent with highest transfection efficiency and intracellular iron content as shown in Figure 2 (PLL; 798.3 +/- 19.0 pixels, PS; 1360 +/- 218.7 pixels, Elp; 661.3 +/- 41.9 pixels, p<0.05).

Conclusion Magnetic labeling of mESC using the 3 transfection methods is safe and effective. Although cellular viability and proliferative capability did not show any difference among the 3 methods, the differentiation capability of mESC transfected by Elp was most attenuated and iron uptake by PS was most efficient.

Reference

1. Arbab AS, Bashaw LA, Miller BR, et al. ***Radiology***. Dec 2003;229(3):838-846.
2. Arbab AS, Yocum GT, Kalish H, et al. ***Blood.*** Aug 15 2004;104(4):1217-1223.
3. Walczak P, Kedziorek DA, Gilad AA, et al. ***Magn Reson Med.*** Oct 2005;54(4):769-774.
4. Yuasa S, Itabashi Y, Ogawa S et al. *Nat Biotechnol* 2005;23(5):607-11

Figure.1 *In Vitro* Bioluminescence Imaging data

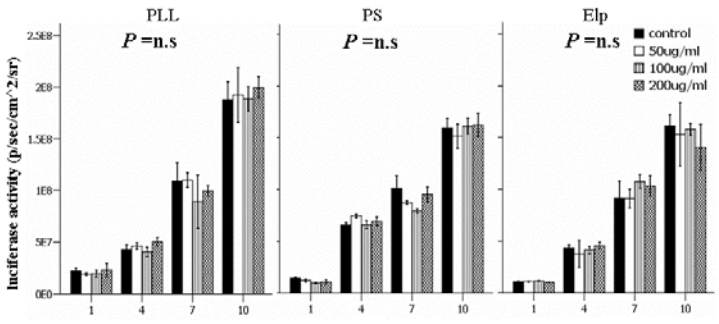

Table.1 MTT assay

	Day1	Day4	Day7	Day14
PLL(50ug/ml),%	96.3±2.1	107.4±8.0	111.6±6.0	113.1±6.4
PLL (100ug/ml),%	98.8±1.1	113.0±4.8	108.0±3.1	106.9±1.7
PLL (200ug/ml),%	87.7±5.6	103.9±1.7	95.0±8.4	109.1±6.2
PS (50ug/ml),%	89.7±3.3	93.2±5.2	109.7±7.7	119.3±1.6
PS (100ug/ml),%	101.2±3.8	104.7±12.1	95.1±6.6	100.3±4.3
PS (200ug/ml),%	91.7±1.4	93.5±5.3	103.7±3.6	117.3±4.4
Elp (2100ug/ml),%	119.0±2.6	123.5±2.1	105.2±2.4	102.9±1.4
Elp (4200ug/ml),%	101.6±6.2	92.4±2.1	103.7±18.8	101.3±1.3
Elp (8400ng/ml),%	106.8±2.3	118.4±6.3	91.2±2.4	96.2±1.9

Data are mean % of the average viability of non-labeled cells

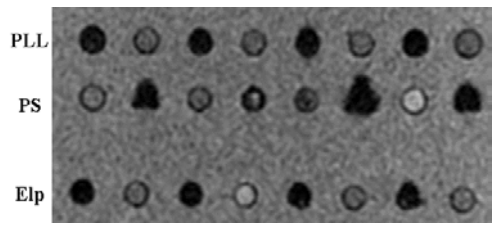

Figure 2. In vitro MRI signal dephasing by ferumoxides labeled 2x10^6 mESC. The largest areas of signal dephasing were measured. Transfection method employing PS demonstrated largest mean area of dephasing (p<0.05).