SPIO labeling promotes cardiac differentiation of embryonic stem cells in vitro but does not prevent their uncontrolled proliferation in vivo

R. Zhou[1], V. Ferrari[2], and H. Qiao[1]

[1]Radiology, University of Pennsylvania, Philadelphia, PA, United States, [2]Medicine (cardiovascular), University of Pennsylvania, Philadelphia, PA, United States

Introduction SPIO and ultra small SPIO particles have a long history in diagnostic imaging for detection of lesions (e.g. cancers) (1) and for angiography (2) respectively. SPIO particles have been utilized for cell tracking via MRI because their intracellular retention facilitates MRI detection of labeled cells in the heart, brain, and other organs. A recent clinical trial in European Union demonstrated for the first time in humans tracking of therapeutic dendritic cells labeled with Feridex (3). When utilized as a diagnostic imaging agent, SPIO particles are introduced intravenously therefore are primarily taken up by reticuloendothelial system, e.g. Kupffer cells, which are able to metabolize the excess iron. Embryonic stem cells (ESCs), however, may not have this capability, therefore effects of iron overloading on cell proliferation and differentiation need to be investigated.

Methods Two types of SPIO particles, nanometer sized Feridex and micrometer sized bangs particles, at 12.5 or 50 μg Fe/ml, were used to label murine ESCs. Cellular iron content was measured by ICP-MASS. Feridex labeled but undifferentiated cells were injected into the infarcted heart and were tracked by MRI over the time course of 4 weeks. Cardiac differentiation was induced in vitro with the hanging drop method (4) and was verified by immunostaining of cardiac markers. Continuous labeling was performed during induction of differentiation to maintain cellular iron content against iron loss due to cell division. Degradation of SPIO was examined by electron microscopy. Viability of labeled ESCs was evaluated using MTT assay at different time points. Percent of total beating EBs were determined over the time course from day 10-19 after hanging drop formation in labeled and unlabeled groups. Western analysis of cardiac specific proteins was also performed to evaluate the extent of cardiac differentiation.

Results Labeling efficiency of 5 pg Fe/cell was achieved at 50 μg Fe/ml for both particles whereas ~2 pg Fe/cell was obtained for Feridex and 1 pg Fe/cell for Bangs particles at 12.5 μg Fe/ml. ESC viability was not affected by labeling with Feridex-poly-L-lysine complex at any time and concentrations examined. Cardiac differentiation in beating EBs was confirmed by immunostaining (Fig1 A-D). Electron microscopy of labeled ESCs show both SPIO particles were degraded in ESCs and strong expression of ferritin, a major iron storage protein, suggest metabolism of SPIOs (Fig. 1E). Cardiac differentiation determined by percentage of total beating EBs and by western blot of cardiac specific proteins was found to be significantly increased by SPIO labeling with both particles. Injection of undifferentiated ESCs in the infarcted or non-infarcted heart, however, resulted in formation of teratoma.

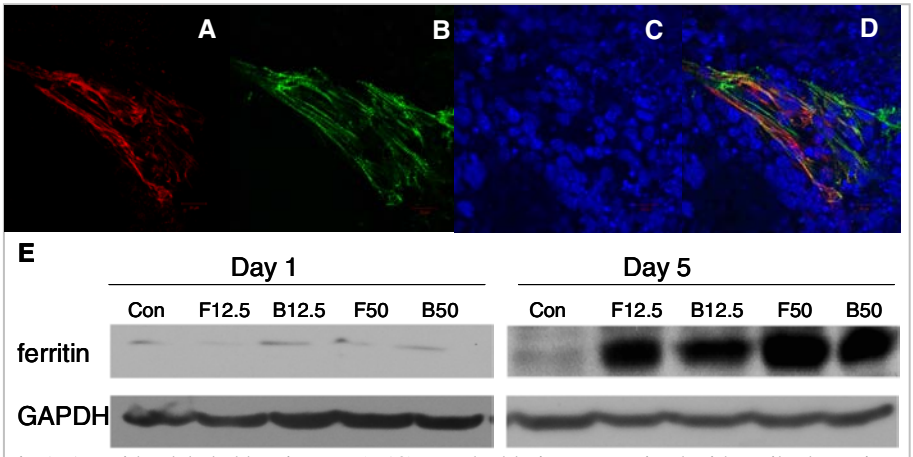

Fig.1. A Feridex labeled beating EB (D19) was doubly immunostained with antibody against troponin I (A) and cardiac -actinin (B). DAPI was used to counterstain the nuclei (C). Overlaid (D) exhibits co-localization of cardiac markers in the beating area. Striations of muscle cells were clearly visible. Western blot analysis of ferritin expression in ESCs at Day1 and in EBs at Day 5 after labeling (E). GAPDH is used as a reference for protein loading.

Discussion Labeling with SPIOs promotes cardiac differentiation of ESCs. However, both infarcted normal myocardiums do not provide enough cues for cardiac differentiation of ESCs.

References 1.Magn Reson Imaging Clin N Am., 1996. **4**: p. 53. 2. J Comput Assist Tomogr., 1996. **20**: p. 51. 3. Nat Biotechnol., 2005. **23**: p. 1407. 4.Circ Res, 2002. **91**: p. 189.