**CELL TRACKING WITH IRON OXIDE LABELLING AND ITS EFFECTS ON MULTILINEAGE DIFFERENTIATION POTENTIAL OF MARROW STROMAL CELLS**

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**INTRODUCTION**

The use of mesenchymal stem cells or marrow stromal cells (MSCs) in vivo both in animal models and in the clinic for the repair of musculoskeletal defects is receiving increasing attention. Knowledge about the fate of these cells following implantation is of critical importance if the safety of stem cell transplantation is to be verified. One method of assessment is cell tracking to observe any undesired cellular migration away from the implant site. Recently, the ability to label single cells with iron oxide particles and visualise them using magnetic resonance imaging (MRI) has been demonstrated. What is not clear however is the extent to which this labeling has an effect on the differentiation capacity of MSCs. The aim of this study was to examine the osteogenic, chondrogenic and adipogenic differentiation potential of adult human MSCs when labelled with superparamagnetic iron oxide (SPIO) in order to assess the long term fate of labelled MSCs following seeding into a tissue engineered scaffold and in vivo implantation.

**METHODS**

Adult human marrow stromal cells were taken during routine surgery with informed consent and in accordance with local ethical guidelines. Cells were expanded and plated onto 6 well plates. Cells were labelled with a concentration of 63.5 μg of iron per well containing 200,000 cells. Transfection of SPIO was achieved using Lipofectamine 2000, which was mixed in equal quantities with the SPIO solution at a final dilution of 1:68 in opti-MEM. After labeling, cells were cultured either in standard expansion medium (DMEM + 10% FCS) or in osteogenic (DMEM + 10mM β-glycerophosphate, 1μM ascorbic acid-2-phosphate), chondrogenic (DMEM + 100μM sodium pyruvate, 40μg/ml prolone, 1:100 ITS, 10ng/ml TGFβ2, 25µ/ml ascorbic acid-2-phosphate) or adipogenic (DMEM + 1µM dexamethasone, 0.2mM, indomethacin, 0.01mg/ml insulin, 0.5mM 3iso-butyl-l-methyl-xanthine) medium for a further 13 days (n=6). After this culture period, cells were harvested for histology, MRI imaging and RT-PCR analysis (n=3, 1 way ANOVA, p<0.05 *).

Labelled cells were also seeded onto collagen glycosaminoglycan (Collagen (GAG) scaffolds for 21 days and then implanted subcutaneously into nude mice for a further 28 days (n=4). Unlabelled cells served as controls (n=5). Following the in vivo period, mice were imaged using a 3T clinical MRI scanner. Scaffolds were subsequently retrieved for histological analysis.

**RESULTS**

MRI images demonstrated that iron uptake into the cells was successful and was retained for 13 days, during which time MSCs differentiated along the different lineages. Prussian blue staining for iron also confirmed the association of iron with the cells and not the surface of the tissue culture dish. Osteogenesis, chondrogenesis and adipogenesis were demonstrated in SPIO labelled cells by histological staining for mineralization, lipid vacuoles and proteoglycans respectively. While there was no apparent difference in the ability of cells to differentiate at the histological level, there were subtle but significant differences in genetic markers when analysed by real-time PCR. Alkaline phosphatase expression was significantly elevated in osteogenically treated cells as expected but not when cells were labelled with SPIO (n=3, 1 way ANOVA). Expression of Sox 9, a chondrogenic marker, was significantly elevated in the chondrogenic condition but expression was further significantly elevated in SPIO labelled cells (n=3, 1 way ANOVA). Fatty acid binding protein 4 (FABP4) expression was also affected by SPIO labeling with a significant drop in expression in adipogenically treated cells containing SPIO compared to unlabelled treated cells (n=3, 1 way ANOVA). Interestingly, there also appeared to be a different pattern of mineralization when cells contained SPIO compared to unlabelled osteogenically treated samples.

**DISCUSSION**

This study demonstrates the ability to label cells with a clinically approved compound and that this compound does not prevent multilineage differentiation of MSCs. Also the long term retention of iron was demonstrated using a clinical MRI machine and confirmed by histological staining for iron. Despite the apparent ability of cells to differentiate while labelled, subtle differences were observed in gene expression in all three lineages and in the pattern of matrix mineralization in osteogenically treated cells. The iron dose used in this study was also approximately 25% of the dose commonly used in cell labeling experiments, demonstrating labeling efficacy even with very low iron quantities but also causing concern about the effect higher doses might be having on cellular activity. It is clear from these results that there is a need for further investigation of the effects of SPIO labeling on differentiation capacity of MSCs. While cells retained the label for 7 weeks and were visible by MRI, this method of labeling will only be of use if it can be demonstrated not to affect normal cellular activities.

**REFERENCES**


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