The effects of iron oxide labelling on the *in vitro* chondrogenic potential of three human cell types

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Introduction: MR imaging has been used to monitor the distribution of administered cells in studies related to the use of cell therapy in regenerative medicine. Labelling cells with Super-Paramagnetic Iron Oxide (SPIO) particles makes it possible to monitor the distribution of these cells within images showing the tissue [1]. There has been disagreement on the effects of this label on cellular differentiation and in particular on development along the chondrogenic lineage [2-3]. Almost all previous studies have employed staining to infer cartilage formation. This study has used the quantitative reverse transcription polymerase chain reaction (qRT-PCR) technique to assess potential affects of the SPIO on chondrogenic gene expression of three different cell types exposed to chondroinductive media for different time periods.

Methods: Measurements were made in Human Bone Marrow Stem Cells (HBMSC), adult chondrocytes and neonatal chondrocytes. All three cell types were labelled with SPIO (Resovist - Schering, Berlin) for 24 hours with an incubation concentration of 0.5 nmol Fe/ml (1µl/ml culture media) at 37^0C and 5% CO_2. A transfection agent was not added and labelling was carried out in a serum free culture medium [4]. After removal of extracellular SPIO, prussian blue staining confirmed the presence of iron within the cells. Chondrogenesis was induced by incubating the cells with a serum free medium consisting of αMEM supplemented with 10 ng/ml TGF-β3, 10^{-8} M dexamethasone, 100µM ascorbate-2-phosphate and 1x ITS supplement. *In vitro* chondrogenesis was examined using RT-PCR for all 3 cell types in both SPIO labelled and unlabelled cells after 2, 7 and 14 days exposure to the chondrogenic media. Relative quantification of Sox9 (*SOX9*), Collagen type II (*COL2A1*) and aggrecan (*ACAN*) gene expression levels in the labelled cells was determined by normalising them to the expression levels in unlabelled cells at the same time point.

Results: Real time RT-PCR revealed differences in expression of *SOX9*, *ACAN* and *COL2A1* both with time and between the cell types. HBMSCs and adult chondrocytes showed normal levels of *ACAN* but a significant relative decrease in the expression of *SOX9* and *COL2A1* in the labelled cells after 2 days culture (Fig 1). After 7 and 14 days these cells showed no significant differences between labelled and unlabelled cells in the expression of *SOX9*, *ACAN* and *COL2A1*. For neonatal chondrocytes, labelling resulted in a decrease in *ACAN* expression after 2 days that recovered to near normal levels by day 7 and then showed another relatively marked decrease after 14 days of exposure to the chondroinductive media.

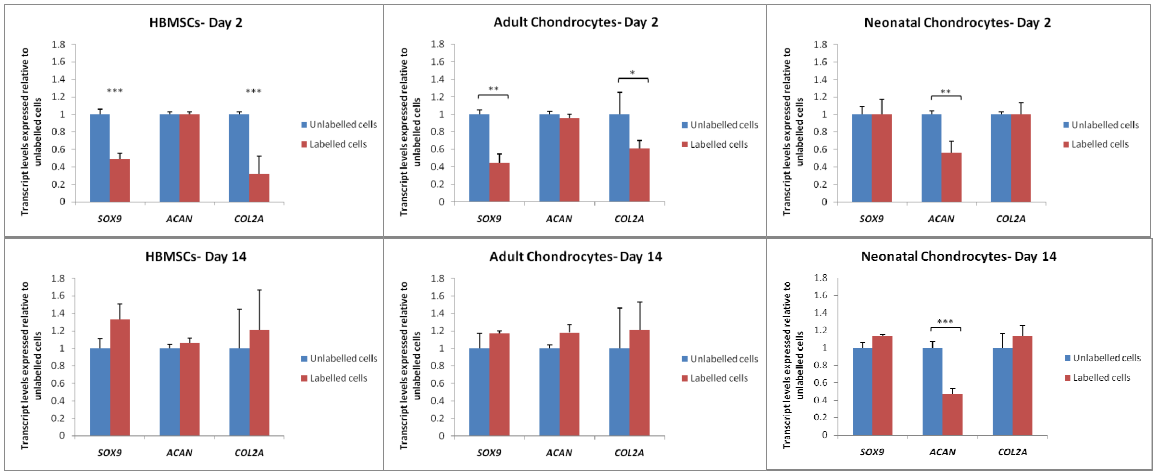

Fig.1: Chondrogenic gene marker expression in HBMSCs, adult- and neonatal-chondrocytes exposed to chondroinductive media for 2 and 14 days. (data for day 7 exposure omitted). This data shows the expression in SPIO labelled cells normalised to that in the unlabelled cells.

Discussion: This study is the first to show that inhibition of gene expression for chondrogenic markers resulting from SPIO labelling is dependent on the target cell used. Whilst HBMSCs and adult chondrocytes show a short term effect due to the label, Resovist does not affect marker gene expression in these cells after approximately 7 days of chondroinductive culture. Neonatal chondrocytes show very different behaviour in that the effect of the Resovist label is still evident on *ACAN* expression at relatively long periods after exposure to the chondroinductive media. The nature of this interference to the chondrogenic pathway in the neonatal cells is unknown. Although this study has not measured the cellular SPIO concentrations, it does indicate that Resovist can be used to label bone marrow stem cells or mature chondrocytes in MR imaging studies of cartilage repair or regeneration.

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[3] Arbab A. et al. Blood 2004; 104:1217. [4] Mailander V et al. Mol Imag Biol 2008; 10:138.