The effects of iron oxide incorporation on the chondrogenic potential of three human cell types

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Abstract

Non-invasive monitoring of living cells in vivo provides an important tool in the development of cell-based therapies in cartilage tissue engineering. High-resolution magnetic resonance imaging (MRI) has been used to monitor target cell populations in vivo. However, the side-effects on cell function of the labelling reagents, such as superparamagnetic iron oxide (SPIO), are still unclear. This study investigated the effect of SPIO particles on the chondrogenic differentiation of human bone marrow stromal cells (HBMSCs), neonatal and adult chondrocytes in vitro. Cells were labelled with SPIO for 24 h and chondrogenesis induced in serum-free medium including TGFβ3. For labelled/unlabelled cells, viability, morphology and proliferation were determined using CellTracker™ Green and PicoGreen dsDNA assays. The expression of SOX9, COL2A1 and ACAN was investigated using qRT–PCR after 2, 7 and 14 days. The results showed that viability was unaffected in all of the cells but cell morphology changed towards a 'stretched' phenotype following SPIO uptake. Cell proliferation was reduced only for labelled neonatal chondrocytes. SOX9 and COL2A1 expression decreased at day 2 but not at days 7 and 14 for labelled HBMSCs and adult chondrocytes; ACAN expression was unaffected. In contrast, SOX9 and COL2A1 expression were unaffected in labelled neonatal chondrocytes but a decrease in ACAN expression was seen at day 14. The results suggest that downregulation of chondrogenic genes associated with SPIO labelling is temporary and target cell-dependent. Resovist™ can be used to label HBMSCs or mature chondrocytes for MR imaging of cells for cartilage tissue engineering. Copyright © 2012 John Wiley & Sons, Ltd.

Keywords cell labelling; superparamagnetic iron oxide; chondrogenic potential; HBMSCs; neonatal chondrocytes; adult chondrocytes; in vitro

1. Introduction

Cell therapy is an emerging tool in regenerative medicine. Bioluminescence, radioactive labelling and near-infrared fluorescence are some of the techniques that have been used to determine cell fate after applications of cell-based therapies by tracking homing and migration of the transplanted cells (Thorne et al., 2006; Contag, 2006; Hofmann et al., 2005; Scarff et al., 2006). The presence of a high proton density in the extracellular matrix (ECM) of articular cartilage allows magnetic resonance imaging (MRI) to non-invasively monitor cartilage degeneration and regeneration in vivo (Jelicks et al., 1993; O’Byrne et al., 2003). Use of an iron-containing ‘label’ results in a relative increase in both the magnetic susceptibility effects and in the MR transverse relaxation rate. The consequence is image hypo-intensity in regions corresponding to the location of the labelled cells that can be detected using gradient-echo imaging techniques (O’Byrne et al., 2003; Hawrylak et al., 1993; Josephson et al., 1999; Kircher et al., 2003; Frank et al., 2003; Dodd et al., 1999; Yeh et al., 1995; Arbab et al., 2004a).
A variety of SPIOs are available for use as MRI contrast agents. Ferumoxides (FE) are a popular dextran-coated SPIO, approved by the US Food and Drug Administration (FDA) for in vivo human use (Arbab et al., 2004a). Dextran-coated SPIO was first used for hepatic MRI (Stark et al., 1988; Weissleder, 1994). Thereafter, a number of studies showed that transfection agents (TAs), such as protamine, poly-L-lysine and lipofectamine, interact electrostatically with the SPIO. These are an effective and stable means to non-covalently bind to DNA and internalize the MR label into the cells (Frank et al., 2003; Kostura et al., 2004). However, the use of TA at high concentrations is said to be toxic, due to electrostatic binding of TA polycations to voltage-gated ion channels, resulting in transient blockage of ion transport (Rink et al., 1994). Thus, the use of SPIO without a TA is held to be a relatively more favorable labeling methodology (Mailander et al., 2008). Recently, a number of SPIO particles that do not require the assistance of TAs, such as Resovist® (generic name, ferucarbotran; Schering, Germany) and Feridex®, have been made commercially available (Mailander et al., 2008). Resovist comprises 10–12 nm magnetic cores stabilized in a carboxydextran biopolymer, which is anionic under physiological conditions (pH 7.4) (Mailander et al., 2008). The overall hydrodynamic diameter of Resovist SPIO particles is 62 nm (measured by photon correlation spectroscopy) and they can increase to a size that is detectable via light microscopy following Prussian blue staining (Mailander et al., 2008; Reimer and Balzer, 2003). The carboxydextran coating prevents aggregation and allows for aqueous solubility of the nanoparticles (Reimer and Balzer, 2003). Mailander et al., (2008) showed Resovist to label cells more efficiently than Feridex without a TA (Mailander et al., 2008). More importantly, Resovist is approved for clinical use in Europe, Japan and Australia (Vogl et al., 1996; Lutz et al., 2005; Wesebe et al., 2006).

Recently, there has been some concern regarding the potential effects of SPIO labelling on cellular chondrogenic differentiation; however, the results so far have been inconsistent (Rink et al., 1994; Butle et al., 2004). The aim of this study was to examine the effect of Resovist® on the chondrogenic potential of human bone marrow stromal cells (representing a progenitor cell population), human neonatal chondrocytes (representing an immature cell population) and human adult chondrocytes (representing a mature cell population) when labelled with Resovist in vitro and to determine whether the inhibitory effects, if any, are target cell-dependent.

2. Materials and methods

Tissue culture flasks and plates were obtained from Nunc™ (Scientific Laboratory Supplies, Nottingham, UK). Passage 1 HBMSCs (obtained from donors aged 38 and 40 years) and adult chondrocytes (obtained from donors aged 35 and 40 years) were obtained from Lonza (Slough, UK). The cells were from haematologically normal donors. Passage 0 neonatal cells were provided by Smith and Nephew (York, UK), with appropriate ethical approval and consent for use in commercial and collaborative research.

2.1. Basic and chondro-inductive cell culture

HBMSCs, neonatal and adult chondrocytes were maintained in basal [a-MEM with 10% fetal calf serum (FCS)] or chondro-inductive media [serum-free a-MEM supplemented with 10 ng/ml TGF-β3 (Peprotech, USA), 10−8 m dexamethasone, 100 µM ascorbate-2-phosphate, 1 × insulin transferring selenium (ITS) supplement (Johnstone et al., 1998; Malpel et al., 2004). The medium was changed every 3 days and cells were passaged at 70–75% confluence. Passage 4 (P4) cells were used for all of the work described here. For chondro-induction, cells were cultured in chondro-inductive medium for 2, 7 and 14 days.

2.2. Magnetic cell labelling

All three cell types were incubated in 0.5 µM Fe/ml Resovist serum-free culture medium at 37°C and 5% CO2 for 24 h (Mailander et al., 2008). The labelled cells were then washed three times with 1× phosphate-buffered saline (PBS; Sigma, UK) to eliminate extracellular SPIO (Ittrich et al., 2007). Control groups for each cell type were cultured under similar conditions but without Resovist.

2.3. Detection of intracellular iron content

Prussian blue staining was used to detect the presence of iron oxide in the cells. Briefly, labelled and unlabelled cells were fixed with 10% neutral buffer formaldehyde. The cells were stained for 30 min with aqueous potassium ferrocyanide (10%) mixed in equal parts with 20% aq. hydrogen chloride (Perl’s Stain Kit, TCS Biosciences, UK) and counterstained in 0.1% nuclear fast red solution for 5 min after three changes of distilled water. The slides were then mounted in p-xylene bis-pyridinium bromide (DPX) and observed under an Olympus BX50 microscope, using SPOT software (version 1.2).

2.4. Evaluation of viability and morphology of iron oxide-labelled cells

The cellular viability and morphology for labelled and unlabelled HBMSCs, neonatal and adult chondrocytes were visually evaluated. Briefly, cells were labelled with CellTracker™ Green CMFDA (5-chloromethylfluorescein diacetate, Invitrogen; 50 µg in 5 ml culture medium) for 30 min and washed thoroughly with serum-free medium twice at 45 min intervals (Partridge et al., 2002; Green et al., 2004; Yang et al., 2006). The cells were then imaged under a fluorescent inverted microscope.
2.5. PicoGreen® dsDNA quantitation assay

Cells were seeded at $4 \times 10^3$ cells/well of each cell type in six-well plates and cultured in basal medium overnight, followed by labelling with or without Resovist ($n = 6$ for both experimental samples and controls), as previously described. After 2, 7 and 14 days of culture in chondrogenic medium, the cells were lysed using 0.1% Triton X-100 and total DNA content was determined using PicoGreen fluorescence reagent, according to the manufacturer’s instructions (Green et al., 2003; Green et al., 2005; Yang et al., 2006). Sample fluorescence was measured using a Fluoroskan Ascent 2.6 microplate reader (excitation 480 nm, emission 520 nm).

2.6. Real-time quantitative reverse-transcription polymerase chain reaction (qRT–PCR)

Resovist®-labelled and unlabelled cells were cultured in T25 flasks for 2, 7 and 14 days in chondrogenic medium ($n = 3$). Total RNA was isolated from the samples using the RNeasy™ mini-kit (Qiagen, UK). RNA (1 µg) was then converted to cDNA using a high-capacity cDNA kit (Applied Biosystems, UK). Real-time RT–PCR was performed (Rotor Gene 6000 Real Time PCR system, Corbett Research, UK). The TaqMan gene expression assay (Applied Biosystems) was used to analyse the expression of chondrogenic markers: Sox9 (SOX9), collagen type II (COL2A1) and aggrecan (ACAN) genes (Grogan et al., 2007; Chubinskaya et al., 1998; Malladi et al., 2006), together with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the housekeeping gene. Our previous publication showed that GAPDH was a reliable housekeeping gene, as its expression levels did not differ between experimental and control samples for the three cell types (Saha et al., 2010). The primer details used for these genes can be found in Table 1. The comparative Ct method ($2^{-\Delta\Delta Ct}$) was employed for quantification of gene expression, in which expression levels of target genes in labelled cells are normalized to the unlabelled controls (Jakob et al., 2001; Afizah et al., 2007; Dyson et al., 2007). An expression level $< 1.0$ in the labelled cells therefore indicates a downregulation of that gene relative to its expression in the unlabelled control (Saha et al., 2010).

2.7. Statistical analysis

Statistical analysis of $2^{-\Delta\Delta Ct}$ values was performed using one-way analysis of variance (ANOVA) with Tukey–Kramer multiple comparisons post-test, using GraphPad Instant Software (GraphPad Software, Inc., San Diego, CA, USA).

3. Results

3.1. Detection of intracellular iron content

After 24 h of incubation with Resovist (0.5 µM Fe/ml), Perl’s Prussian blue staining revealed cytoplasmic uptake of the iron oxide nanoparticles for all three cell types, as indicated by positive blue-stained intracellular vesicles (Figure 1), although the amount of staining varied. Non-labelled cells for all three cell types were unstained by Perl’s Prussian blue.

3.2. Effect of SPIO labelling on cell viability

Resovist labelling had no observable adverse effects on cell viability when compared to non-labelled controls for all three cell types, as evidenced by staining with CMFDA (Figure 2). The fluorescent CMFDA labelling also permitted visualization of cell morphology. This suggested that labelling might be associated with changes to cell morphology, especially in the case of labelled neonatal and adult chondrocyte cell groups, where the cells appeared to be more stretched out and ‘stringy’ in comparison to the unlabelled cells. However, this preliminary observation would need to be confirmed using quantitative histomorphometric data in future studies.

3.3. Effect of SPIO labelling on cell proliferation

There was no significant difference in cell proliferation between labelled and unlabelled HBMSCs and adult chondrocytes at any of the three time points (Figure 3). However, a highly significant decrease in the proliferation of labelled neonatal chondrocyte cells was observed in comparison to unlabelled control cells after 2 days of culture ($p < 0.001$). After 7 days, there was no significant difference in proliferation between labelled and unlabelled neonatal chondrocytes ($p > 0.05$). After 14 days, labelled neonatal chondrocytes were observed to have a significantly higher proliferation rate than the control cells ($p < 0.05$).

### Table 1. Primers used in TaqMan gene expression assays

| Gene   | Ref. sequence  | Assay ID          | Exon boundary | Assay location | Amplicon length |
|--------|----------------|-------------------|---------------|----------------|-----------------|
| GAPDH  | NM_002046.3    | Hs99999905_m1     | 3–3           | 154            | 122             |
| SOX9   | NM_000346.3    | Hs00165814_m1     | 2–3           | 1060           | 102             |
| COL2A1 | NM_033150.2    | Hs00264051_m1     | 6–7           | 503            | 124             |
| ACAN   | NM_013227.3    | Hs00153936_m1     | 11–12         | 2644           | 91              |

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Figure 1. Perl’s Prussian blue (PPB) staining of HBMSCs, neonatal and adult chondrocytes. Light micrographs showing: (A–C) unlabelled cells; (D–F) Resovist-labelled cells. Blue stain indicates successful cell labelling with Resovist after 24 h. The unlabelled cells were unstained by PPB and only stained for Congo red. Magnification, ×10

Figure 2. Effect of SPIO labelling on cell morphology. Both labelled and unlabelled cells for each cell type were observed to be viable (stained fluorescent green with CFMDA). However, the morphology of labelled neonatal and adult chondrocytes appeared to be more stretched out and flattened in comparison to the unlabelled samples for each cell type. HBMSCs, human bone marrow stromal cells; NC, neonatal chondrocytes; AC, adult chondrocytes
3.4. Effect of Resovist labelling on chondrogenic gene expression

qRT–PCR revealed temporal differences in expression of SOX9, ACAN and COL2A1 between labelled and unlabelled cells for all three cell types under chondrogenic culture conditions.

For HBMSCs, a significant decrease in the expression of SOX9 and COL2A1 was observed in the labelled cells after 2 days of culture compared to unlabelled cells (p < 0.01), while the expression levels of ACAN appeared to be unaffected by the labelling. However, after 7 and 14 days, the expression levels for SOX9, ACAN and COL2A1 were not significantly different between labelled and unlabelled cells (Figure 4).

For neonatal chondrocytes, there was a significant (p < 0.01) downregulation of SOX9 and COL2A1 gene expression in the labelled group compared to the unlabelled group after 7 days of culture in chondrogenic medium, but no significant differences were detected at days 2 and 14 (Figure 5). A significant decrease in the expression of ACAN was observed in the labelled cells after 2 and 14 days of chondrogenic culture compared to unlabelled controls. However, there were no significant differences at day 7 (Figure 5).

In the case of adult chondrocytes, SPIO labelling resulted in a similar chondrogenic gene marker expression to that of HBMSCs (Figure 6). A statistically significant decrease in expression levels of SOX9 and COL2A1 in the labelled group was observed after 2 days of culture in comparison to the unlabelled group, followed by no significant differences in expression levels of all three genes at days 7 and 14.

4. Discussion

SPIO labelling of cells is a popular technique employed as a non-toxic and non-invasive method for monitoring cells and cell therapy using MR imaging (Dodd et al., 1999; Ittrich et al., 2007; Lewin et al., 2000; Hoehn et al., 2002). There has been a debate around the labelling of cells with SPIO and its possible effect(s) on cell differentiation (Arbab et al., 2004a; Kostura et al., 2004; Bulte et al., 2004). Our study on the effects of SPIO labelling on chondrogenesis was carried out using three different human cell types and used gene expression as a measure of labelling impact on chondrogenic phenotype, rather than cell staining or the detection of cell surface markers.

Arbab et al. (2004) labelled MSCs with ferumoxides, using protamine sulphate as the TA, and showed via histological staining that chondrogenesis was not inhibited (Arbab et al., 2004a). In a later report, Arbab et al. (2004b) demonstrated similar Alcian blue staining in labelled and unlabelled MSCs, suggesting that ferumoxides did not inhibit chondrogenesis. They also stated that chondrogenic inhibition could arise due to differences in the transfection agent (TA) used or as a result of incomplete washing to remove the iron oxide–TA complexes (Arbab et al., 2004b). In contrast, Kostura et al. (2004) showed a marked inhibition of chondrogenesis upon labelling MSCs with Feridex coupled to poly-L-lysine as the TA. In their study, chondrogenic inhibition was mediated solely by the SPIO used and was independent of the type of TA employed (Kostura et al., 2004). While these studies looked at the possible effects of using an SPIO that required a TA on MSCs, there is still uncertainty about whether the effects of SPIO labelling are dependent on target cell type. In this study, the effects of Resovist without the use of a TA have been investigated on the chondrogenic potential of cells from different sources that might be used for cartilage repair (e.g. HBMSCs, human neonatal chondrocytes and human adult chondrocytes) (Saha et al., 2010).

This study confirmed labelling of the three different human cell types after a simple 24 h incubation of the cells with Resovist (0.5 μM SPIO/1 ml culture medium) without the use of a TA. A serum-free labelling protocol was used in this study, as it has been suggested that SPIO nanoparticles form conglomerates with serum which adhere strongly to the cells’ surfaces and which cannot be easily removed (Mailander et al., 2008). The cell labelling used here was easily detected via MRI and differences between labelled and unlabelled cells (negative staining) in the decay of transverse magnetization (T2) (data not shown).

Our results showed that cell labelling did not appear to alter the viability of any of the three cell types, which is similar to the findings of others (Stark et al., 1988; Mailander et al., 2008; Bulte et al., 2004). Our data also suggested a possible change in cell morphology after labelling. Although this remains to be confirmed in future work, Farrell et al. (2008) also reported a phenotypic change in Feridex–labelled MSCs in vivo, similar to that seen during endochondral ossification (Farrell et al., 2008), and suggested that SPIO labelling inhibited osteogenic but not chondrogenic differentiation of MSCs.

In the present study, Resovist labelling was observed to significantly decrease cell proliferation rates compared to unlabelled control cells only in the neonatal chondrocyte group at the earliest time point used (day 2). At day 14, enhanced proliferation of the labelled cells was detected. It is possible that the enhanced proliferation rate of labelled neonatal chondrocytes at day 14 days may be a compensatory mechanism following the initial downregulation.

Inhibition of chondrogenic gene expression seen after Resovist labelling appeared to be a short-term effect only in the case of HBMSCs and adult chondrocytes. This contradicts previous studies showing the inhibitory effects of SPIO labelling on MSC chondrogenic differentiation (Kostura et al., 2004; Bulte et al., 2004; Farrell et al., 2008). It is possible that the short-term downregulation of chondrogenic genes observed here at day 2 for labelled HBMSCs and
adult chondrocytes is related to effects of the label on the membrane-bound transferrin-like protein (MTf), a glycosylphosphatidylinositol anchored protein expressed at high levels by chondrocytes (Oda et al., 2003). MTf upon crosslinking with Concanavalin-A, results in a change in cell shape and leads to chondrogenic induction (Oda et al., 2003). Expression patterns of MTf are said to parallel that of collagen type II with the onset of chondrogenesis (Nakamasu et al., 1999). It has been reported that cellular divisions over time lead to a step wise decrease in the number of SPIO positive cells (Yano et al., 2005). Thus, between day 2 of chondrogenesis and day 7, the SPIO concentration should have been diluted considerably, perhaps allowing MTf to resume normal morphology and activity to maintain normal chondrogenic gene marker expression levels. Schafer et al., (2009) also observed non inhibition of chondrogenic differentiation upon labelling HBMSCs with Resovist® after 14 days of chondrogenic culture (Schafer et al., 2009).

A significant decrease in expression levels of ACAN by labelled neonatal chondrocytes was observed in comparison to the control cells at days 2 and 14 in chondrogenic culture. ACAN expression levels were seen to recover in the labelled cells at day 7. However SOX9 and COL2A1 expression levels were significantly decreased in the labelled cell population at day 7. Our study therefore shows downregulation of key chondrogenic markers after intracellular incorporation of Resovist to be target cell-dependent. The mechanism of downregulation of chondrogenic gene expression by Resovist in neonatal chondrocytes is unknown. A difference in the pattern of temporal expression of chondrogenic gene markers was previously observed between human neonatal chondrocytes and human adult HBMSCs/chondrocytes (Saha et al., 2010). It is possible that the Resovist inhibitory effect observed in neonatal chondrocytes at day 14 could be due to intrinsic differences in their signalling pathways compared to adult cells. Different morphological zones in adult/mature cartilage tissue release a variety of bioactive molecules, such as lubricin and cartilage intermediate layer protein (CILP) (Niikura and Reddi, 2007; Lorenzo et al.,

Figure 3. Effect of Resovist labelling on cell proliferation. A significant difference in cell proliferation between labelled and unlabelled cells was observed only in the neonatal chondrocyte group. Results are expressed as mean ± SD (n = 3): *p < 0.05; **p < 0.001

Figure 4. Effect of Resovist labelling on chondrogenic gene expression by HBMSCs. Resovist labelling affected expression levels of SOX9 and COL2A1 during the initial stages (day 2) in chondrogenic culture. Gene expression levels were then similar for labelled and unlabelled cells at days 7 and 14. Results are expressed as mean ± SD (n = 3): *p < 0.05; **p < 0.01; ***p < 0.001
1998), while numerous proteins are involved in forming ECM trans-activating complexes with SOX9 (SOX5/SOX6, CREB/p300 and c-Maf) (Lefebvre et al., 1998; Tsuda et al., 2003; Huang et al., 2002). These molecules may be capable of reducing/removing any interference that Resovist may have on the intracellular signalling processes.

Human neonatal cartilage is also known to contain two types of chondrocytes (Hwang, 1978). The lack of relevant information regarding the purpose and differences between these two types of chondrocytes makes it difficult to infer how Resovist might affect chondrogenesis in these cells. Further studies on the exact mechanism of chondrogenesis and the different biomolecules involved are warranted, as this study suggested that a 0.5 μM Fe/ml addition of Resovist cannot be used to label human neonatal chondrocytes, due to its effects on the mechanism of chondrogenic differentiation. We note that our use of the comparative Ct method cannot determine threshold levels of gene expression which would impact on the relevant protein expression.

It is possible that the observed short-term effects of labelling are related to a heat shock response to the cellular uptake of Resovist, although this hypothesis remains to be tested. Heat shock protein (HSP) expression can be induced by stress factors, such as heavy metals or reactive oxygen intermediates (Winrow et al., 1990; Schett et al., 1998), and HSP70 and HSP32 are known to be expressed in chondrocytes under stress (Kaarniranta et al., 2001). A high expression of HSP70 in cartilage has been shown to reduce proteoglycan synthesis and induce chondrocyte apoptosis (Sawatzky et al., 2005). However, low levels of HSP70 and HSP32 have been shown to be chondroprotective (Sawatzky et al., 2005).

The determination of the absolute amounts of SPIO taken up by the cells would have been a useful measure to inform further discussion as to the effects of labelling.
The observed differences in the effects of the label on chondrogenesis and cell morphology and proliferation could reflect dissimilar amounts of the iron oxide label taken up within the different cell types.

5. Conclusion

This study observed that Resovist at 0.5 μM Fe/ml was incorporated by HBMSCs, neonatal and adult chondrocytes without affecting cell viability. We have also demonstrated, for the first time, that downregulation of chondrogenic gene expression with Resovist is apparently only a transient effect that is target cell-dependent. These results are noteworthy, as Resovist could be used at this concentration as a useful tool for non-invasive monitoring of stem cells and mature chondrocytes in vitro and in vivo for cartilage repair/regeneration.

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References

Aizah H, Yang Z, Hui JH et al. 2007; A comparison between the chondrogenic potential of human bone marrow stem cells (BMSCs) and adipose-derived stem cells (ADSCs) taken from the same donors. Tissue Eng 13: 659–666.

Arbab AS, Yocum GT, Kalish H et al. 2004a; Efficient magnetic cell labelling with protamine sulfate complexed to ferumoxides for cellular MRI. Blood 104: 1217–1223.

Arbab AS, Yocum GT, Kalish H et al. 2004b; Feride-protamine sulfate labelling does not alter differentiation of mesenchymal stem cells. Blood 104: 3412–3413.

Bulte JW, Kraitchman DL, MacKay GT et al. 2004; Chondrogenic differentiation of mesenchymal stem cells is inhibited after magnetic labelling with ferumoxides. Blood 104: 3410–3413.

Contag CH 2006; Molecular imaging using visible light to reveal biological changes in the brain. Neuroimaging Clin N Am 16: 633–654.

Chubinskaya S, Huch K, Schulze M et al. 1998; Human articular chondrocytes cultured in alginate beads maintain their gene expression. Cells and Material 8: 151–160.

Dodd SJ, Williams M, Suhan JP et al. 1999; Detection of single mammalian cells by high resolution magnetic resonance imaging. J Biophs 76: 103–109.

Dyson JA, Genever PG, Dalgarno KW et al. 2007; Development of custom-built bone scaffolds using mesenchymal stem cells andapatite-wollastonite glass-ceramics. Tissue Eng 13: 2891–901.

Farrell E, Wielopolski P, Pavljusev P, et al. 2008; Effects of iron oxide incorporation for long term cell tracking on MSC differentiation in vitro and in vivo. Biochem Bioiphys Res Commun 369: 1076–1081.

Frank JA, Miller BR, Arbab AS et al. 2003; Clinically applicable labelling of mammalian and stem cells by combining superparamagnetic iron oxides and transfection agents. Radiology 228: 480–487.

Green D, Haward D, Yang XB et al. 2003; Natural marine sponge fibre skeleton: a biomimetic scaffold for human osteoprogenitor cell attachment, growth and differentiation. Tissue Eng 9: 1159–1166.

Green D, Walsh D, Yang XB et al. 2004; Stimulation of human bone marrow stromal cells using growth factor encapsulated calcium carbonate porous micropores. J Mater Chem 14: 2206–2212.

Green D, Leveque I, Walsh D et al. 2005; Bio-mineralized polysaccharide capsules for encapsulation, organization, and delivery of human cell types and growth factors. Adv Funct Mater 15: 917–923.

Grogan SP, Barbero A, Diaz-Romero J et al. 2007; Identification of markers to characterize and sort human articular chondrocytes with enhanced in vitro chondrogenic capacity. Arthritis Rheum 56: 586–595.

Hawrylak N, Gosh P, Broadus J et al. 1993; Nuclear magnetic resonance (NMR) imaging of iron oxide labelled neural transplants. Exp Neurol 121: 181–192.

Hoehn M, Kustermann E, Blunk J et al. 2002; Monitoring of implanted stem cell migration in vivo: a highly resolved in vivo magnetic resonance imaging investigation of experimental stroke in rat. Proc Natl Acad Sci USA 99: 16267–16272.

Hofmann M, Wollert KC, Meyer GP et al. 2005; Monitoring of bone marrow cell homing into the infarcted human myocardium. Circulation 111: 2198–2202.

Huang W, Lu N, Eberspaecher H, 1978; Ultrastructure of human foetal and neonatal hyaline cartilage. J Pathol 114: 568–573.

Hwang WS, Jakob M, Demarteau O, Schafer D et al. 2003; Monitoring at 3 T. J Magn Reson Imaging 160: 1191–1197.

Kamiranta K, Holmberg CI, Lammi MJ et al. 2001; Primary chondrocytes resist hydrostatic pressure-induced stress while primary synovial cells and fibroblasts show modified HSP70 response. Osteoarthritis Cartil 9: 7–13.

Kircher MF, Allport JR, Moore A et al. 1993; In vivo high resolution three-dimensional imaging of antigen specific cytotoxic T lymphocyte trafficking to tumors. Cancer Res 63: 6838–6846.

Kostura I, Kraitchman DL, MacKay AM et al. 2004; Feridex labelling of mesenchymal stem cells inhibits chondrogenesis but not adiopogensis or osteogenesis. NMR Biomed 17: 513–517.

Lefebvre V, Li P, De Crombrugghe B. 1998; A new long form of Sox 5 (L-Sox5), Sox 6 and Sox9 are coexpressed in chondrogenesis and cooperatively activate the type II collagen gene. EMBO J 17: 5718–5733.

Lewin M, Carlesso N, Tung CH et al. 2000; Tat peptide derivatized magnetic nanoparticles allow in vivo tracking and recovery of progenitor cells. Nat Biotechnol 18: 410–414.

Lorenzo P, Bayliss MT, Heinegard D. 1998; A novel cartilage protein (CILP) present in human articular cartilage increases with age. J Biol Chem 273: 23463–23468.

Lutz AM, Willmann JK, Goepfert K et al. 2005; Hepatocellular carcinoma in cirrhosis: enhancement patterns at dynamic gadolinium- and superparamagnetic iron oxide-enhanced T1-weighted MR imaging. Radiology 237: 520–528.

Mailänder V, Lorenz MR, Holzapfel V et al. 2008; Carboxylated superparamagnetic iron oxide particles label cells intracellularly without transfection agents. Mol Imaging Biol 10: 138–146.

Malladi P, Xu Y, Chou M et al. 2006; Effect of reduced oxygen tension on chondrogenesis and osteogenesis in adipose-derived mesenchymal cells. Am J Physiol Cell Physiol 290: C1139–C1146.
Effects of SPIO labelling on cell chondrogenesis

Reimer P, Randazzo N, Cancrissa R et al. 2004; Serum free growth medium sustains commitment of human articular chondrocyte through maintenance of Sox9 expression. Tissue Eng 10: 145–155.

Nakamasu K, Kawamoto T, Shen M et al. 1999; Membrane-bound transferrin-like protein (MTf): structure, evolution and selective expression during chondrogenic differentiation of mouse embryonic cells. Biochem Biophys Acta 1447: 258–264.

Niikura T, Reddi AH. 2007; Differential regulation of lubricin/superficial zone protein by transforming growth factor-β1/bone morphogenetic protein superfamily members in articular chondrocytes and synoviocytes. Arthritis Rheum 56: 2312–2321.

O’Byrne E, Pellis T, Laurent D. 2003; Qualitative and quantitative in vivo assessment of articular cartilage using magnetic resonance imaging. In Tissue Engineering of Cartilage and Bone, Bock G, Goode J (eds), Novartis Foundation Symposium 249. Wiley: Chichester.

Oda R, Suardita K, Fujimoto K et al. 2003; Anti-membrane-bound transferrin-like protein antibodies induce cell-shape change and chondrocyte differentiation in the presence or absence of concanavalin A. J Cell Sci 116: 2029–2038.

Partridge K, Yang XB, Clarke NMP et al. 2002; Adenoviral BMP-2 gene transfer in mesenchymal stem cells: in vitro and in vivo bone formation on biodegradable polymer scaffolds. Biochem Biophys Res Commun 292: 144–152.

Reimer P, Balzer T. 2003; Ferucarbotran (Resovist): a new clinically approved RES-specific contrast agent for contrast-enhanced MRI of the liver: properties, clinical development, and applications. Eur Radiol 13: 1266–1276.

Rink T, Bartel H, Jung G et al. 1994; Effects of polycations on ion channels formed by neutral and negatively charged alamethicins. Eur Biophys J 23: 155–165.

Saha S, Kirkham J, Wood D et al. 2010; Comparative study of the chondrogenic potential of human bone marrow stromal cells, neonatal chondrocytes and adult chondrocytes. Biochem Biophys Res Commun 401: 333–338.

Sawatzky DA, Foster R, Seed MP et al. 2005; Heat-shock proteins and their role in chondrocyte protection, an application for autologous transplantation. Inflammopharmacology 12: 569–581.

Scarf M, Arnold SA, Harvey LM, et al. 2006; Near infrared spectroscopy for bioprocess monitoring and control: current status and future trends. Crit Rev Biotechnol 26: 17–39.

Scaffer R, Kehlbach R, Müller M et al. 2009; Labeling of human mesenchymal stromal cells with superparamagnetic iron oxide leads to a decrease in migration capacity and colony formation ability. Cytotherapy 11: 68–78.

Schett G, Redlinck C, Xu Q et al. 1998; Enhanced expression of heat shock protein 70 (HSP70) and heat shock factor 1 (HSF1) activation in rheumatoid arthritis synovial tissue. J Clin Invest 102: 302–311.

Stark DD, Weissleder D, Elizondo G et al. 1988; Superparamagnetic iron oxide: clinical application as a contrast agent for MR imaging of the liver. Radiology 168: 297–301.

Thorne SH, Negrin RS, Contag CH. 2006; Synergistic antitumor effects of immune cell-viral biotherapy. Science 311: 1780–1784.

Tsudo M, Takahashi S, Takahashi Y et al. 2003; Transcriptional Co-activators CREB-binding protein and p300 regulate chondrocyte-specific gene expression via association with Sox9. J Biol Chem 278: 27224–27229.

Vogl TJ, Hammenstingl R, Schwartz W et al. 1996; Magnetic resonance imaging of focal liver lesions. Comparison of the superparamagnetic iron oxide Resovist versus gadolinium–DTPA in the same patient. Invest Radiol 31: 696–708.

Weissleder R. 1994; Liver MR imaging with iron oxides: toward consensus and clinical practice. Radiology 193: 593–595.

Wensebe A, Wiskirchen J, Decker U et al. 2006; Comparison of gadolinium–BOPTA and ferucarbotran-enhanced three-dimensional T1-weighted dynamic liver magnetic resonance imaging in the same patient. Invest Radiol 41: 264–271.

Winrow YR, McLean L, Morris CJ, et al. 1990; The heat shock protein response and its role in inflammatory disease. Ann Rheum Dis 49: 128–132.

Yang XB, Webb D, Blaker J et al. 2006; Evaluation of human bone marrow stromal cell growth on biodegradable polymer/Bioglass® composites. Biochem Biophys Res Commun 342: 1098–1107.

Yano S, Kuroda S, Shichinohe H et al. 2005; Do bone marrow stromal cells proliferate after transplantation into mice cerebral infarct? A double labeling study. Brain Res 1065: 60–67.

Yeh TC, Zhang W, Ildstad ST et al. 1995; In vivo dynamic MRI tracking of rat T-cells labelled with superparamagnetic iron oxide particles. Magn Reson Med 33: 200–208.