# Bifunctional iron oxide nanoparticles for MR imaging and hyperthermia therapy in cancer

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**Introduction:**
Superparamagnetic iron oxide nanoparticles (SPIO) have diverse applications in biomedicine, and have recently been used for cell tracking of mesenchymal stem cells (MSCs) within the body using MRI [1]. MSCs are easily labelled with SPIO, with little effect on cell proliferation or survival [2]. These MSCs will home to and incorporate themselves within lung metastases *in vivo* after systemic administration, and can hence be used as a delivery vector to carry SPIO into tumour sites deep within the body [3]. This homing and delivery can be visualised with MRI. SPIO particles can also provide an anti-tumour therapy with the application of an alternating magnetic field (AMF). The AMF causes rapid and sustained heating of the SPIO and causes damage to surrounding cancer cells, which is the basis of hyperthermia therapy for cancer [4,5]. MRI can be used before and after hyperthermia treatments to monitor SPIO distribution within the tumour. In this study we investigate the potential of SPIO as bifunctional nanoparticles for hyperthermia treatment in a subcutaneous murine tumour model and the possibility of MR monitoring of SPIO labelled MSCs within the tumour.

**Methods:**
*MSCs & tumour model*: MSCs were purchased from Tulane University, New Orleans and were cultured in α-MEM/16% FBS with L-glut (4mM) & pen/strep. (50units/ml / 50µg/ml). MSCs were incubated overnight with 0.5mg/ml of the SPIO Resovist for labelling & were co-labelled with cell-tracker CM-DiI. OVCAR-3 human ovarian cancer cells were cultured in DMEM/8% FBS with L-glut & pen/strep. Subcutaneous tumours were induced by co-injecting $0.5x10^6$ MSCs & $2x10^6$ OVCAR cells into the flank of female Balb/c nude mice aged 6 weeks.
*MRI*: Images were obtained on a 9.4T horizontal bore Varian NMR system using a 39mm RF coil. A fast spin-echo sequence was used to evaluate the presence of SPIO.
*Hyperthermia therapy:* A 3 turn copper solenoid coil of diameter 3cm was used to create a 10mT 1.05MHz AMF. Mice were anaesthetised throughout with Isofluorane and tumour surface & core rectal temperatures were monitored throughout with fibre optic probes. An infra-red thermal imaging camera allowed monitoring of overall tumour temperature compared with mouse body temperature. Hyperthermia sessions lasted 20 minutes and each mouse in the treatment group received 4 sessions spaced 48 hours apart. Control mice received 20 minutes of Isofluorane in the coil with the current switched off.
*Histology:* Post-hyperthermia histology was obtained to evaluate the presence and distribution of SPIO labelled MSCs, and subsequently compared with that seen with MRI.

**Results:**
Labelling MSCs with Resovist had no effect on cell proliferation or survival (data not shown). SPIO labelled MSCs could be visualised on MR images before and after hyperthermia treatments as a region of hypointensity (Fig. 1-A, B, E, F). The magnitude of the hypointensity caused by these SPIO labelled MSCs decreased after hyperthermia treatments (Fig. 1-B). Fibre optic temperature probes and infra-red thermal imaging confirmed a temperature rise in the tumour of 4°C above core body temperature during hyperthermia treatments (Fig 1- I). After hyperthermia treatments, SPIO labelled MSCs were confirmed within the tumour histologically with Prussian blue staining of tumour sections (Fig. 1-C, G). H&E staining revealed areas of necrosis after hyperthermia treatment and confirmed that the SPIO labelled MSCs were located mainly in tumour margins (Fig. 1-D), as seen on MR imaging (Fig. 1-B, F).

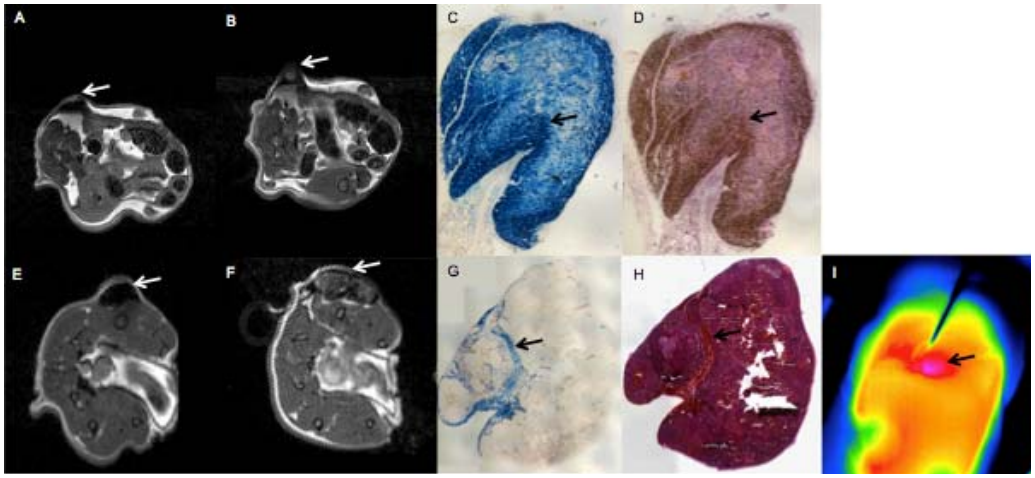


Figure 1: A- fsems of control tumour (arrowed) at day 14 post cell injection. B- fsems of tumour (arrowed) day 22 after injection. C- Prussian blue stain of control tumour showing the presence of SPIO throughout (arrowed). D- consecutive H&E stain in the same tumour. E- fsems of tumour (arrowed) in the treatment group prior to the first hyperthermia treatment. F- fsems of the same tumour after 4 hyperthermia treatments. G- Prussian blue showing SPIO in the treated tumour. H- H&E stain of a consecutive section in the same tumour. I- infra-red thermal imaging picture of the same mouse receiving hyperthermia treatment (tumour arrowed).

**Discussion and Conclusion:**
In this study we have demonstrated the potential of SPIO as a bifunctional MR contrast agent and cancer therapy. We have shown hyperthermia treatment using SPIO labelled MSCs increases tumour temperature leading to cell death. We have also shown that MR imaging can be used to non-invasively confirm the presence of SPIO within tumours 2 weeks after MSC co-injection. In addition, MR images showing the position of SPIO labelled MSCs within tumours correlates with Prussian blue staining in histology sections.

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**References:** [1] Loebinger *et al*. Cancer Research 2009, 1;69(23):8862-7, [2] Farrell et al. Biochem Biophys Res Commun 2008 16;369(4):1076-81, [3] Karp *et al*. Cell Stem Cell 2009 4(3):206-216 [4] Gazeau *et al*. Nanomedicine 2008, 3(6):831-44, [5] Saito *et al*. Cancer Science 2008, 99(4):805-9.