FERUMOXIDE LABELLING OF CD133+ CELLS FOR TARGETED RE-ENDOTHELIALISATION

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Introduction

Labelling of cells with MRI contrast agents is becoming an established method of tracking cells *in vivo* in animals and has recently entered the clinical setting (1). It may also be possible to exploit the magnetic properties of these particles to apply an external magnetic force on the labelled cells both *in vitro* and *in vivo* (2,3). The long term aims of our study are to monitor the homing and retention of endothelial progenitor cells (EPCs) non-invasively using MRI and target them to the site of injury using an externally applied magnetic field, thus enhancing re-endothelialisation.

EPCs are proliferative cells that can adopt an endothelial-like phenotype. Although the exact identity of the EPCs remains elusive, they are known to be involved in pathological and physiological processes such as tumour-induced and post-ischaemic neovascularisation, and vascular re-endothelialisation. Recent evidence suggests that EPCs express the early haematopoietic ('angioblast') marker CD133, as well as the CD34 and VEGFR2 markers (4,5,6). Our group has studied CD133+ derived progenitor cells acquired from peripheral blood in vascular repair. Cultivation of CD133+ cells for 10 days in pro-angiogenic conditions ("d10-CD133") results in maturation to committed cells that become adherent, express endothelial markers (VE-Cadherin, VEGF receptors) and are able to engraft into the de-endothelialised carotid artery after balloon angioplasty in an animal model.

We have previously highlighted a difference in iron uptake between the adherent and suspension fractions of the d10-CD133 cells (7), which we have surmounted using a differential labelling technique in this study. Here we present intial results of the viability and functional assessment of these cells (with/out magnetic actuation), MRI assessment after labelling, quantification of iron uptake and computer modelling of the magnetic forces on ferumoxide particles.

Methods

Cell culture and labelling: Human CD133+ cells were isolated from apheresis samples of GCSF-stimulated donors and cultured on fibronectin-coated plates for 10 days as previously described (7). For differential labelling with Endorem (Guerbet, 500ugFe/ml), the suspension d9-CD133 fraction was separated and labelled for 23h followed by additional labelling for 1h with the adherent fraction (24:1 labelling). For standard labelling the whole cell population was labelled for 24 h (24:24 labelling). Iron uptake was quantified with a superconducting quantum interference device.

Magnetic actuation: NdFeB disk magnets, dia 22mm x 8mm, grade N35SH (Magnet Apps Ltd) were used for the viability experiments. These were placed for 24h underneath the 4 corner wells of a 96-well plate, each containing $3x10^4$ labelled d10-CD133s 1mm from the magnet surface. Magnetic actuation was directly followed by the MTS assay (below). The magnet array for the *in-vivo* study was made using 5mm x 5mm x 25mm N35 NdFeB magnet blocks (Fig 4).

Viability and functional assays: The MTS assay (Promega) was used for cell viability analysis; 490nm absorbance was measured at 4h. Three experimental groups, each with 4 samples of $3x10^4$ cells, were examined: a) 24:24 labelling without magnet b) 24:24 with magnet c) 24:1 with magnet. Functionality analysis for the Endorem-labelled d10-CD133+ cells stained with CellTracker Dye (Invitrogen) was done with a matrigel assay. $4x10^4$ d10-CD133s, labelled with Endorem or unlabelled, were co-cultured with $4x10^4$ HUVECs on matrigel in EGM-2 for 12 hours.

MRI: To assess MRI visibility of d10-CD133s after 24:1 ferumoxide labelling, $5x10^5$ 24:1 labelled d10-CD133s were pelleted in a 250ul eppendorf. $5x10^5$ unlabelled CD34+ cells were pelleted similarly as a control. Images were taken on a 2.35T horizontal bore SMIS system using a 2DFT spin-echo sequence (30mm FOV, 128x128 matrix, TR=2s, TE=70ms, 2mm slice, 2 averages).
Computer modelling: Vectorfields 3D (Opera) was used to model the magnets and resulting forces on the Endorem particles.

Results

Fig 1a, b shows that standard labelling (24:24) of d10-CD133s results in decreased viability leading to cell lysis after application of a magnetic force corresponding to 0.5 fN (0.5e-15 N) per Endorem particle (Fig 1b). Fig 1c demonstrates that 24:1 labelled cells are not compromised by the magnetic force. These differentially labelled cells contain, on average, 2.5 pg Fe/cell (~4.7e+4 particles), which is sufficient to cause loss of signal in a standard spin-echo sequence (Fig 2b) compared to control (Fig 2a). Importantly, labelled and unlabelled d10-CD133s have similar functionality as demonstrated using the matrigel assay (Fig 3). Finally, specific magnet arrays have been modelled and developed to maximise and homogenise the applied force for the upcoming *in-vivo* magnetic targeting experiments (Fig 4).

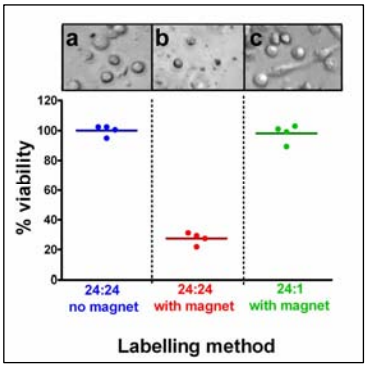

Figure 1: Viability assessment (MTT assay).

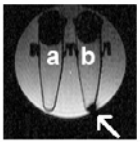

Figure 2: MRI visibility
a: Unlabelled control cells
b: 24:1 labelled d10-CD133
TR=2s, TE= 70ms

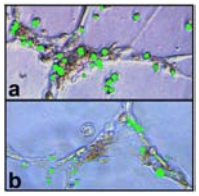

Figure 3: Matrigel assay
Unlabelled d10-CD133 (a) and labelled d10-CD133 (b) are attached to the endothelial (HUVEC) network. d10-CD133 are shown as green.

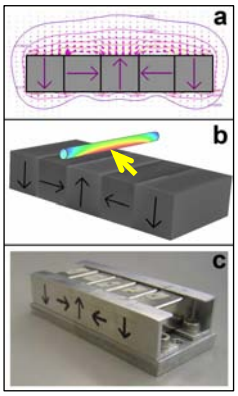

Figure 4: Magnet array
a)The arrows represent the direction of magnetisation of each block. Such a configuration concentrates the field and force to one side of the array.

b)The array will exert a uniform force along the artery (arrow, max 2 fN per Endorem particle).

c) Photograph of the constructed magnet array.

Discussion

Very limited information is available regarding the effect of magnetic actuation on ferumoxide-labelled cells. Here we show that significant cell death can be caused, and is most likely due to mechanical strain. A method to label the suspension and adhesive cell fractions differentially was developed to maintain cell viability. Iron uptake was sufficient for cell visualisation with a low-field (2.35T) MRI system. A preliminary qualitative test to assess functionality showed no differences between labelled and non-labelled d10-CD133s in incorporating into an endothelial network. Finally, computer modelling has been used to improve magnet design for *in vivo* experiments. Using a multidisciplinary approach we have provided *in vitro* evidence for cell viability after labelling and magnetic actuation, and demonstrated the feasibility of *in vivo* targeting of stem cells to a site of cardiovascular injury and their visualisation using MRI.

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