MAGNETIC TARGETING OF STEM CELLS TO A SITE OF VASCULAR INJURY USING AN MRI CONTRAST AGENT

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Introduction: Labelling of cells with iron oxide 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 by imposing 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 and are known to be involved in pathological and physiological processes such as vascular re-endothelialisation and post-ischaemic neovascularisation (4,5,6). Cultivation of CD133+ progenitor 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 and the potential negative impact of magnetic actuation on viability (7). We have surmounted this using a differential labelling technique, which also readily allows for MRI visibility of a cell pellet using a 2.35T system (8). Here we present further results of the viability assessment of these cells, MRI visibility, preliminary results from our magnetic targeting *in vivo* study and finally data from an *in vitro* system for the magnetic capture of cells in flow.

Methods

Cell culture and labelling: Human CD133+ cells were cultured 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).

Magnetic actuation and viability: NdFeB disk magnets grade N35SH were placed for 24h underneath culture wells containing labelled d10-CD133s. Magnetic actuation was directly followed by an Annexin-V apoptosis assay at 24h, 48h and 120h (below) and an MTS viability assay at 24 hours (not shown). The magnet array for the *in-vivo* study was made using 5mm x 5mm x 25mm N35 NdFeB magnet blocks in *Halbach* configuration.

MRI visibility: Single cell suspensions of a) $150x10^3$/mL, b) $15x10^3$/mL and c) no cells (control) were prepared 0.5% w/v low melting point agarose at room temperature and were scanned in 250uL PCR eppendorf tubes (end vol. 330uL). Images were acquired using a 9.4T horizontal bore Varian VNMRS system and a 2D gradient-echo sequence (30mm FOV, 1024x1024 matrix, 0.4mm slc, TR=400ms, TE=11ms, FA=20, 6 av).

Animal model: Balloon angioplasty was performed on the left common carotid artery of male Sprague-Dawley rats. This was followed by administration of iron-labelled d10-CD133s in the absence of flow with and without an external magnetic field (total n=12). Arteries were exposed at 24 hours, dissected and confocal microscopy was performed. Quantification of cell adhesion for each group is under way.

Results: Fig 1 shows that at 24 hours following magnetic actuation of differentially labelled d10-CD133s there is no increase in cell apoptosis. Similarly, there is no increase at 48h and 120h following actuation. Furthermore, the iron oxide labelling itself did not cause an increase in apoptosis (not shown). Figure 2 demonstrates that labelled cells can be visualised in an agarose phantom at concentrations fourfold lower than those observed in our *in vivo* study (Fig 2b). Specific magnet arrays have been modelled and constructed to maximise and homogenise the applied force for the *in-vivo* magnetic targeting study (not shown). Most importantly, preliminary *in vivo* and *in vitro* data indicate increased EPC adhesion to the injured artery (Fig. 3a) and to the tubing in a 1mL/min flow phantom (Fig. 3b) following magnetic targeting.

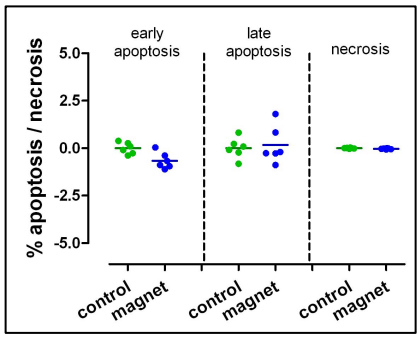

Figure 1: Annexin-V apoptosis assay

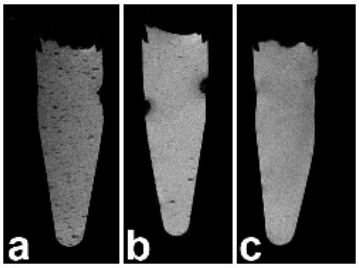

Figure 2: MRI visibility
a: 50.000 cells per eppendorf
b: 5.000 cells per eppendorf
c: control

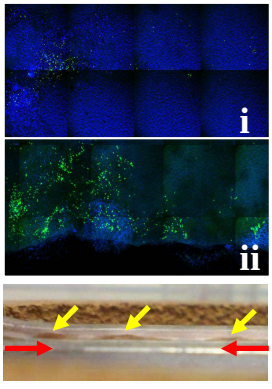

Figure 3a: *In vivo* targeting Sample en face confocal microscopy images of arterial lumen from i) control and ii) magnetic targeting group.

There are increased numbers of adhering cells (green) in the targeted group, max 2000 per artery.

Figure 3b: Flow phantom. Side view of 1mm tube with cells captured in flow (yellow arrows) using the magnet array (red arrows).

Discussion: A method of labelling the suspension and adhesive cell fractions differentially has been developed to maintain cell viability following magnetic actuation. Here we have provided *in vitro* evidence for cell viability following labelling and magnetic actuation, as well as MR visualisation at low cell concentrations. Computer modelling has improved magnet design for *in vivo* experiments, and our preliminary *in vivo* results indicate that *in vivo* targeting using an externally applied magnetic field is a promising approach to increase engraftment of stem cells to a site of vascular injury.

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