Targeting stem cells following i.v.injection using magnetic particle based approaches

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The success of using stem cell-based therapies for tissue repair and regeneration requires a suitable type and source of stem cells, the ability to deliver and track the cells *in vivo*, and effective mechanisms for targeting the cells to the site of tissue injury. Human mesenchymal stem cells (MSCs), a population of adult stem cells present in a multitude of tissues, offer significant therapeutic potential owing to their capacity to differentiate into numerous mesodermal tissues including bone, cartilage, muscle and adipose. They can readily be isolated from the bone marrow and adipose tissue and have substantial expansion potential *ex vivo,* whilst maintaining multipotentiality. MSCs are also immune privileged cells, possess immunoregulatory properties and can home to sites of inflammation *in vivo*. Nevertheless, the efficacy of using stem-cell based therapies first relies on the ability to specifically track and target these cells to sites of tissue injury.

The specific targeting of cells to sites of tissue damage *in vivo* is a major challenge precluding the success of stem cell-based therapies. Magnetic particles, widely used in a variety of biomedical applications, may provide a solution. Magnetic particles, ranging in size from a few nanometres to several microns, have been utilized in a variety of biomedical applications, including as contrast agents in magnetic resonance imaging, for targeted drug delivery and hyperthermia treatment *in vivo*, for cell separation and sorting *in vitro*, and recently for tissue engineering and targeting strategies. They are characteristically of low or non-toxicity, are biocompatible and magnetic (superparamagnetic in the case of iron oxide particles), and can be fabricated in a highly controlled manner, allowing for diverse functionalities according to the application.

These properties provide a foundation upon which magnetic particle labelling of cells may be used to specifically target cells *in vivo* using magnetic fields following injection. Little data exists however describing the targeting potential of magnetic particle labelled MSCs *in vitro* or *in vivo*. We have been using in vitro and in vivo experimental approaches to determine the potential for using magnetic particles to label human MSCs and targeting of these during fluid flow using a magnetic field

To examine cell trapping using magnetic particles, we designed a simple *in vitro* fluid flow system incorporating a magnetic trapping chamber. Experiments using this system demonstrated that MSCs labelled with 250 nm diameter magnetic particles could be specifically trapped during fluid flow, with a calculated trapping efficiency of 33%. This was based on taking a single ‘snapshot’ of trapping during flow (flow rate 3.0 ml/minute), and held true for cells that passed within 0.1 mm of the tubing inner surface. Whilst the majority of cells in flow were not trapped as they passed the magnet, continuous flow would lead to increased trapping and accumulation of cells. Indeed, this was evident in our *in vitro* experiments where cell trapping occurred quickly after initiation of flow (within 30 seconds) and increased substantially with continuous flow. We next assessed the effect of particle concentration and fluid flow rate on the extent of cell trapping. We were then able to study the effects of other

parameters such as presence of protein, blood cells and viscosities on the ability to target and trap stem cells within our experimental chamber.

Using principles established *in vitro*, a proof of concept *in vivo* experiment using magnetically labeled, luciferase transfected MSCs were trapped by an implanted magnet in a subcutaneous wound model in nude mice. Magnetic particle labeled human ASCs were injected into the tail vein, tracking the bioluminescent signal non-invasively using a CCD camera. As a control, one animal was injected with luciferase transfected human ASCs without magnetic particle labeling. Whole animal imaging on Day 1 revealed a distinct region of intense localized signal on the left lateral side of the dorsal surface in the mouse injected with magnetically labeled cells (NM1), at the exact site of the implanted magnet. No equivalent signal was detected on the right lateral side where the collagen fleece control was implanted, nor was any signal detected at either site in the animal injected with non-magnetic ASCs (NM2). Similarly no signal in the region of the lungs was detected. Surgical removal of the magnet and collagen fleece control had no discernable affect on the signal localized to the site of the magnet in the mouse injected with magnetically labeled ASCs or the absence of signal in the animal injected with non-magnetic ASCs. Hence, MSCs targeted to the magnet were retained at this site even after withdrawal of the magnetic field. Our *in vivo* cell targeting data are supported by the work of Kyrtatos et al (2009), who report the *in vivo* targeting of endothelial progenitors to the site of vascular injury using the clinically approved superparamagnetic iron oxide particle Endorem (Guerbet). In their approach, magnetically labelled progenitor cells were injected directly into the site of arterial injury and were retained here by the use of a magnetic actuator. Whereas, we use a more systemic approach, injecting the cells into the tail vein and delivering these via the circulation to the site of a magnet in the mid-dorsal region.

Our results provide a model for testing superparamagnetic iron oxide particles to define successful trapping of MSCs during fluid flow which ultimately can be translated to *in vivo* targeted delivery of cells via the circulation in a variety of tissue repair models.

The study group is asked to help us to better understand

- the mechanics of the fluid flow and magnetic field, and how they influence the trajectories and trapping of the injected cells and particles. Ideally, we hope to have a mathematical model of our *in vitro* system which would help us validate our experimental results.
- the sensitivity of multiple parameters in the vascular system of a patient (e.g., blood flow rates, viscosity, etc.) in relation to the delivery to a specific tissue.
- the limitations of using magnetic fields for directed targeting to specific organs in terms of location, depth of body and size of repair site.
- the degree of dosing of magnetic particles and strength of magnetic field for different applications within a patient

Experimental data will be provided at the study group.