Magnetic Resonance Imaging of Iron Labelling of Mesenchymal Stem Cells Seeded on 3-D Type I Collagen and in Gels

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Aim: Visualisation and tracking of USPIO labelled Mesenchymal stem cells (MSCs) in collagen gels and scaffolds by ex vivo MR imaging.

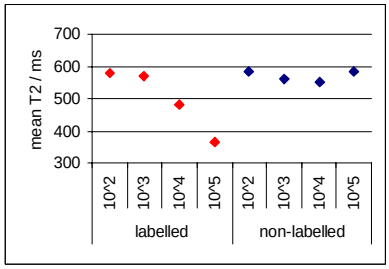
Background: Mesenchymal stem cells, with their potential for rapid proliferation and differentiation into various cell types are a promising candidate cell for tissue engineering heart valves. In order to track cells after implantation, an effective and clinically applicable way of labelling these cells *in vivo* is important.[1] USPIO labelled MSCs have been visualized *in vivo* after implantation in the myocardium of animals.[2] However, the efficiency of this technique for visualization of cells seeded on tissue engineered valve scaffolds has not yet been tested.

Methods: Human MSCs were labelled with iron by incubating for 48 hours with superparamagnetic iron oxide using poly-L-lysine as transfecting agent. Gels of evenly dispersed cells at concentrations up to $1x10^5$ were studied. These gel samples were approximately 15mm diameter and $1cm^3$ in volume. For the scaffolds, $4.5x10^5$ labelled cells were seeded onto 1% Type I bovine collagen scaffolds (diameter of 13mm and thickness of 1.5mm). Control scaffolds used non-labelled cells.

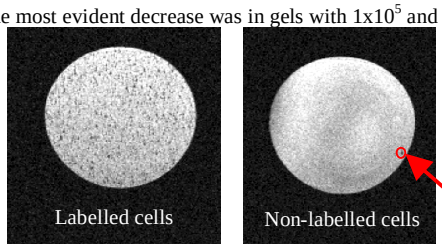
MR scanning was performed with a Siemens 1.5 T scanner. Scans were carried out at baseline and once per week for the following four weeks. T2 measurements were made of the gels as this will be characteristic of the iron concentration. Typical parameters for a multislice spin-echo sequence were repetition time 2000 ms and echo times of 60 - 600 ms for quantification of T2. Images had a matrix size of 128 x 128, 3 slices (each 0.8mm thick) and a 60mm field of view with two signal averages. A high resolution gradient echo (GRE) image of the gels with labelled and unlabelled cells at the highest concentration had parameters TR/TE 160/25.2ms, FOV 67x50, matrix 1024x768, 32 slices of thickness 0.17mm and 10 averages.
For the scaffolds, images were acquired using a 3D GRE sequence. In order to get the best quality images, and also to test what could be achieved in a shorter scan time, two different approaches were used: (i) A single short scan: 2.4 hours, transverse orientation with post processing reconstruction (MPR) of the orthogonal planes; (ii) Scanning overnight, which allows higher SNR scans in both transverse and coronal orientations separately.
Imaging parameters: (i) tranverse, TR 100ms, TE 10ms, FOV 60x56.25mm, matrix size 256, 52 slices, voxel size 0.23x0.23x0.35mm, 7 averages, flip angle 25°, scan time 2.4 hours. (ii) tranverse, TR 100ms, TE 10ms, FOV 60x60mm, matrix size 256, 52 slices, voxel size 0.23x0.23x0.35mm, 24 averages, flip angle 25°, scan time 8.8 hours. This can also be used for a coronal reconstruction in the same way as scan (i), but a separate coronal scan was also acquired with parameters: TR 100ms, TE 10ms, FOV 67x50.25mm, matrix size 128, 56 slices, voxel size 0.52x0.52x0.17mm, 32 averages, flip angle 25°, scan time 4.7 hours. In all cases it was easier to compare a consistent 3D MPR of coronal images from the transverse with 40 slices each 0.2 mm to cover the sample. This gives tranverse and reconstructed coronal images of the same resolutions from both overnight and 2.4 hour scans, the only difference being the number of signal averages. Scans were carried out at baseline and once per week with scan (i) for weeks 0, 2 and 4 and scan (ii) in weeks 1 and 3.

At the same time, any detrimental effect of the iron labelling on cells was determined by MTS assays (number of viable cells) and Lactate Dehydrogenase (LDH) detection (cell death). Finally, Prussian blue staining, confocal microscopy and electron microscopy were used in order to visualize USPIOs within the cells.

Results:
Gels: The presence of iron labelling in sufficient concentration decreased T2. The most evident decrease was in gels with $1x10^5$ and $1x10^4$ labelled cells.

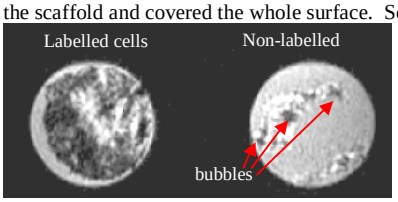


The graph shows the mean T2 values over the 4 weeks for each concentration of labelled and non-labelled cells in gel.

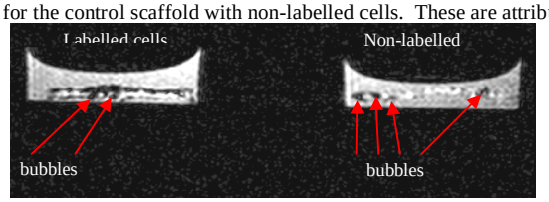


A high resolution scan showed individual signal voids due to the presence of cells containing USPIO labelling.

Scaffolds: Scaffolds seeded with labelled cells showed hypointense signal regions in T1 weighted images, in comparison with the respective controls. In transverse images, the area of reduced intensity covered the top surface of the scaffold, reflecting homogeneous seeding. Both short and long duration scans were effective in visualizing the labelled cells. Labelling remained effective even at scans performed 4 weeks after the labelling procedure. Signal from region containing scaffold is slightly brighter than the surrounding media which aids the visualisation of cell migration in relation to the scaffold. Over 4 weeks the cells migrate down the edges of the scaffold and covered the whole surface. Some black regions are also visible for the control scaffold with non-labelled cells. These are attributed to air bubbles.



Coronal reconstruction (upper surface of scaffolds)



Transverse image of scaffolds after 4 weeks

For validation, the presence of intracellular iron in the labelled cells was shown by Prussian blue staining. In addition, confocal microscopy with the use of the reflectance channel revealed reflecting particles within the cytoplasm of labelled cells only. Electron microscopy revealed the presence of multiple intracellular vesicles containing dark material exclusively in the labelled cells, whereas no such intracellular formations were detected in non-labelled cells of same passage. Cell viability and proliferation were comparable in both labelled and non-labelled cells, indicating there was no detrimental effect due to either the USPIO or the labelling technique.

Conclusion: USPIO labelling of human MSCs seeded on collagen scaffolds is an effective technique for visualization of these cells by MR imaging when they are in high enough concentration. This technique appears very promising for the *in vivo* tracking of cells and in future clinical applications such as tissue engineered valves.

[1] JA Frank, BR Miller, AS Arbab, et al. *Radiology*, 2003; 228: 480 - 487.s
[2] JM Hill, AJ Dick, VK Raman, et al. *Circulation*. 2003;108:1009-1014