Investigating DNP markers for stem cell tracking at low field

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Introduction:

There is increasing interest in studying the fate of stem cells in living organisms as they are used as putative therapeutic agents for a range of diseases. However, improved sensitivity MR visible cell markers are required to track the small cell volumes used for in vivo studies. This abstract reports initial work aimed at producing a stem cell marker and imaging system for in vivo studies. Dynamic nuclear polarisation (DNP) or Overhauser MRI is a remarkable method for increasing the imaging sensitivity using biologically stable free radicals such as 3-Carbomyl-PROXYL with possible enhancement factors of up to two orders of magnitude in real time [1-3]. As the imaging frequency increases, the electron spin resonance frequency also increases in proportion but the microwave penetration depth decreases. In vivo studies are best performed at low frequency (<300 MHz ESR) where penetration effects are less and SAR is reduced. This study investigated the use of a relatively low field (8.2 mT, 348 kHz proton imaging frequency) dedicated MR system [4] for DNP where the corresponding ESR frequency is 239 MHz.

Fig. 1: DNP coil

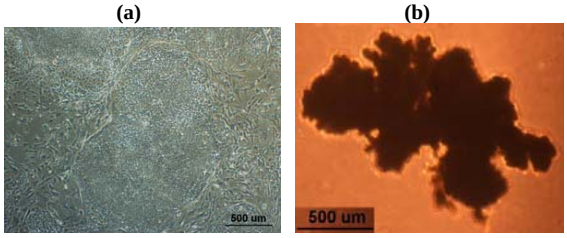
Methods:

The MR system was supplied with 16.1 Amp current from a DC power supply (Agilent 6673A, U.S.). A split solenoid transmit-receive coil (30 mm diameter, 65 mm length, 11 mm gap in the middle) was used for NMR imaging, and was tuned to 348 kHz. Another RF loop coil (30 mm diameter) was placed at the centre of the split solenoid to produce the ESR irradiation (Fig.1). The EPR transmit coil was tuned to 239 MHz and supplied by a 50W LZY-1 Power Amplifier (Mini-Circuit Europe, U.K.). The spin probe, 3-Carbamoyl-Proxyl, was prepared in distilled water at a concentration of 3 mM, which has been reported to give an optimum DNP enhancement [3]. The phantom was a cylindrical vial with a cap (28 mm diameter, 70 mm length) filled with ~40 ml C-Proxyl solution.

The first experiment was performed using the phantom with NMR imaging and either with or without ESR irradiation at a range of T_{ESR} (10-750 ms). The second experiment was performed with both NMR and EPR (at T_{ESR} = 500 ms) for a range of ESR powers (-10 to 6 dBm input to amplifier). The first and second experiments were performed without applying any gradients. Then, a DNP imaging experiment (at T_{ESR} = 500 ms and ESR power = 6 dBm input to amplifier) was performed for a plant transpiration study. The cap of the phantom was opened, and a daffodil stem was inserted into the solution with the flower still attached outside the coil. A coronal plane in the middle of the phantom was acquired every hour for six hours within a single day with another two scans on the next day. The signal from the daffodil stem was calculated for each image. Typical scan parameters for a gradient-echo sequence were TR/TE = 100/5 ms, ESR irradiation time (T_{ESR}) = 10-750 ms, number of averages = 5, phase-encoding steps = 128, number of samples = 256, slice thickness = 3 mm, FOV = 140 mm. The data analysis was performed using Matlab 7.0 (The MathWorks Inc., MA).

Fig. 2(a): Colonies of human embryonic stem cells (Shef 4 cell line). (b) Embryoid body (EB).

(a) **(b)**

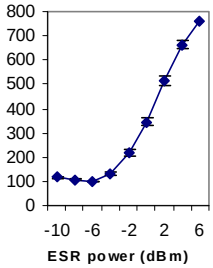


Following these DNP imaging feasibility studies, human embryonic stem cells (Shef 4 cell line, Fig.2a) were prepared *in vitro* for 5 days as embryoid bodies (EBs) to a diameter of ~1 mm^3 (Fig.2b). Ten to twenty EBs were immersed overnight in 3 mM C-Proxyl in culture medium in a 15 mm diameter vial which was placed inside the ESR coil during imaging. Following imaging, the cells were removed from the C-Proxyl medium, washed and then imaged again in stem cell culture medium. Images were acquired using the same gradient-echo sequence above with and without CW ESR irradiation (at 239MHz) for both cases.

Results:

In the first experiment with ESR irradiation, the signal amplitude of the phantom increased with the T_{ESR}, and it reached a plateau at ~500 ms. The measurement without ESR irradiation was used as a control which did not show any significant increase in signal with T_{ESR}. The result from the second experiment is plotted in Fig.3, which shows the increase of signal amplitude with the increase of ESR power at T_{ESR} = 500 ms showing an overall enhancement of around 8x with the maximum available power. Fig.4 is the set of images acquired from the flower stem using DNP every hour arranged from left to right. It was observed that the signal within the stem increased significantly after 6 hours which is assumed to be the time for the C-Proxyl radical to travel 35 mm up the stem. The last two images were acquired on the next day, where the stem had slightly moved from its original position but the signal enhancement was still clearly seen.

Fig.3: Signal amplitude versus ESR power at T_{ESR} = 500 ms.



In the stem cell tracking without ESR irradiation, no image intensity was seen whereas Fig.5 shows the stem cells in C-Proxyl and culture medium after applying ESR irradiation. The image was noisy and the stem cell cluster locations were not clearly visualised. DNP imaging on the washed stem cells in pure culture medium could not produce any image. No significant enhancement corresponding to the stem cell clusters was thus observed.

Fig.4: Transpiration of 3-carbamoyl-PROXYL solution in a plant stem using DNP.

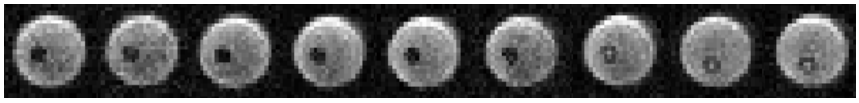
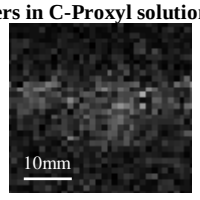


Fig. 5: DNP image of stem cell clusters in C-Proxyl solution.



Discussion & Conclusion:

Experiments on a low field MR system have demonstrated the feasibility of imaging plant transpiration of heavier molecules than water and showing that DNP is feasible using the 8.2 mT system. Stem cells have the potential to provide reparative strategies for a range of diseases but in vivo studies are being hampered by the lack of suitable imaging markers to visualise the path of the cells as they target different organs in the body. We have investigated using a low field imaging system for imaging stem cells marked with a DNP tracer. Thus far no enhancement has been observed from the stem cell clusters but this is believed to be due to the low number of stem cell clusters and thus large partial volume effect used for these experiments. There is also a possibility that the C-Proxyl may not have crossed the cell membranes or that the C-Proxyl had reacted with the culture medium. The plant studies suggest that, with a relatively low molecular weight (MW=189), C-Proxyl should be taken up by cells. A larger volume of cells (0.5 mL) is currently being cultured to test whether the negative result was in fact due to partial volume effects. An improved design of DNP coil is currently under development, which consists of a saddle coil for EPR and a Helmholtz coil for NMR, which is expected to improve the SNR and signal uniformity.

References: [1] Lurie et.al. J.Magn.Reson. 1988; 76: 366-370. [2] Utsumi et. al. PNAS 2006; 103:1463-1468. [3] Benial et. al. JMR 2006; 182: 273-282. [4] Krjukov et. al. Conc.Magn.Reson.Part B 2007; 31B: 209-217.