Gd-emulsion particles for monitoring stem cell therapies: relaxivity and cellular uptake

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Purpose/Introduction:

Recent findings have proven that MRI is an excellent, non-invasive tool for tracking transplanted neural stem cells (SC) labelled with an iron-based contrast agent (CA) [1]. Gadolinium could be more desirable as a CA for the purpose of SC-labelling as it will allow to further monitor tissue regeneration using other MRI measurements (MT, diffusion) following the SC grafting. Gd-based labelling has had moderate success, but has not been as effective for monitoring the migratory progress of cells [2]. The purpose of this study was to develop novel, gadolinium-based CAs appropriate to label stem cells (SC) prior to transplantation into damaged or diseased tissue, and provide improved contrast enhancement for SC therapy monitoring.

Methods:

We have produced a gadolinium-loaded, emulsion-based particle that is both an MRI contrast agent (CA) and a lipofection agent making it appropriate for labelling cells. This CA is prepared by initial combination of biocompatible surfactants and a lipophillic fluorophore, solvation of this mixture in de-ionized water, addition of the hydrophobic core substance and, lastly, high-powered homogenization (Fig. 1). The surfactants employed are lecithin, cetyltrimethylammonium bromide (CTAB) and Gd-DTPA covalently linked to bis(linolelyamide) that incorporate both gadolinium and cationic charge into the outer shell. The average size of the CAs was measured with a dynamic light scattering method (Malvern Instruments, Worchestershire, UK). Quantification of the gadolinium incorporated into the CA was evaluated with x-ray k-fluorescence spectroscopy. The x-ray k-fluorescence data was used for calculating both the amount of gadolinium within cells labeled with the emulsion CAs. T_1 and T_2 relaxivities were measured with a 30 cm horizontal bore superconducting magnet (Nalorac, Martinez, CA) operating at 1.5 T and equipped with a spectroscopy console (SMIS, Surrey, England).

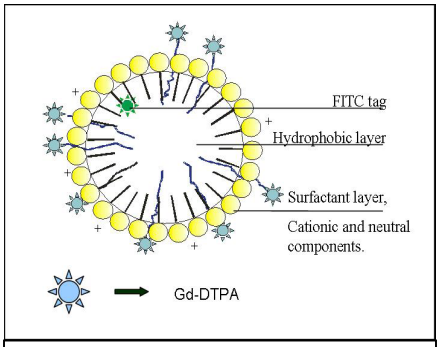

Fig.1 Schematic representation of the emulsion-based contrast agent.

We used murine embryonic stem cell (ESC) line. Cellular labelling was mediated simply by mixing the CA with transfection medium and incubating cells in this medium. The cationic charge made the particles conducive to cellular uptake via endocytosis. Positive identification of cellular CA uptake was visualized with fluorescence microscopy and further quantified using the Xenogen IVIS system (Xenogen Corporation, USA) providing fluorescent quantitative analysis. Initial fluorescence intensity of CA was correlated to the concentration of gadolinium present prior to cellular treatment. Following cellular uptake, the intensity of the fluorescent signal allowed for determination of the amount of gadolinium internalized by the SCs. Following cell labelling with CA, serial dilutions of cells were suspended in a 0.1% agarose in PBS solution for MR measurements.

Results:

The emulsion CAs had a T_1 relaxivity of 8-11$(mMs)^{-1}$ at 1.5T, depending on preparation. Cellular uptake of these CAs proved to be highly efficient (Fig. 2) based on fluorescence spectroscopy, quantitation of fluorescence and MR measurements (Table 1). The fluorescent intensity of treated cells increased with higher treatment concentration as compared to controls. Pronounced decreases in T_1 and T_2 relaxation times of cell suspensions were also observed and allow for detection of $5x10^5$ ESCs in suspension. By plotting the fluorescent emission of the CAs vs. the measured Gd concentration (Fig.3) it was possible to correlate the intensity of the fluorescence emitted by the labeled ESCs to average internal Gd concentrations.

Treatment: time (hours) and concentration of Gd (mM)	Number of Cells	T_1 Relaxation (s)	T_2 Relaxation (s)
Control	10^6	2.2	0.93
12 hours, 0.004mM	10^6	1.74	0.86
Control	$5x10^5$	2.34	1.3
12 hours, 0.007mM	$5x10^5$	1.6	0.78

Table 1: T_1 and T_2 relaxation times of embryonic stem cells suspended in 0.1% agarose-PBS solution following treatment with different concentrations of the emulsion CA.

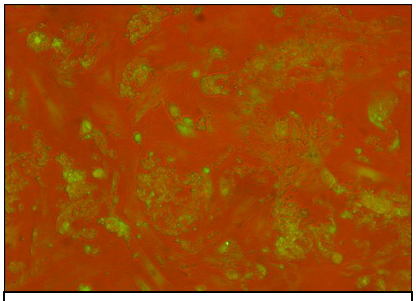

Fig. 2: 100x magnification with a FITC filter of ESCs following incubation with the cationic CA. All colonies exhibit fluorescence.

Conclusion:

The emulsion particles are novel in this application as CAs and are ideal due to the ease of cellular uptake and high T_1 relaxivity. The correlation of the fluorescence to Gd concentration provides a method in order to estimate the amount of Gd within the labelled cells. The detectability of the labelled cells provides promising insight that these novel CAs will allow for an effective method for tracking SC migration in *in vivo* trials. Due to the non-specific nature of CA uptake, we predict this will be an effective method to label and track a number of different cell types.

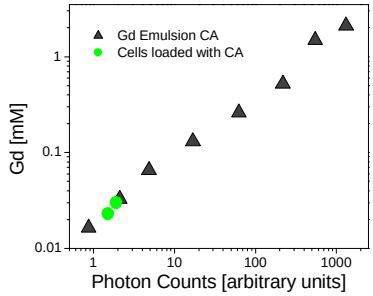

Figure 3: A log-log plot of the fluorescent intensity (photon counts) of the emulsion CA vs. the Gd concentration (mM).

References:

1. Bulte JWM [2003] *et al. Magn Reson Med* 50:201–205.
2. Modo M [2004] *et al. Neuroimage* 21:311-317.