Theoretical considerations on the quantification of iron oxide labeled cells in vivo

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Introduction

Iron oxide contrast-enhanced MRI has become a commonly used tool in molecular and cellular imaging. By labeling cells with iron oxide nanoparticles, it is possible to create a strong local magnetic field inhomogeneity. This leads to signal attenuations in T2 and T2* weighted MR images on the site of the cell. These hypointense spots can be utilized on longitudinal studies to sensitively track labeled cells, providing a powerful non-invasive tool for biological questions. Besides other problems such as ambiguity of the signal attenuation, another major issue which is not successfully solved until now is the quantification of these labeled cells. Due to the insufficient resolution of MRI, only strongly labeled and sparsely distributed cells can be detected separately. This allows quantification of cell numbers by direct counting. For denser packing and lower cell labels, the measurement of T2 and T2* is suggested for quantification [1-4]. All of these studies, however, suffer from the unknown amount of iron oxide internalized by the cells during *in vivo* situations. To circumvent this problem two approaches are used. Either, the total iron amount per volume is quantified [1, 2, 4] or calibration curves are used to determine the cell density from the relaxation times [3]. These calibration curves must be determined by *in vitro* experiments or histological studies. One possible solution to avoid calibration curves is the measurement of both relaxation times. Another one is measuring the dependence of the T2 relaxation time on the inter-echo time of a multi spin echo sequence. In this work we investigate these possibilities by studying theoretical models for the transverse relaxation in the presence of diffusion on the typical parameter range of *in vivo* situations.

Materials and methods

The difficulty of cell quantification by a single measurement results from the problem that two unknown parameters influence the relaxation times. These parameters are the cell density and the iron amount internalized by the cells. Thus, several combinations of these parameters will result in the same relaxation time, which can be visualized by the contour lines in a 2D plot (cf. Fig. 1). If, however, these contour lines for different measurements vary from each other, only one or a very few parameter combinations are allowed for a particular combination of the transverse relaxation times. This might allow the determination of both parameters simultaneously. In this work we utilize the model of Ziener et al. [5] for the T2* relaxation time and the model of Jensen et al. [6] for the multi-echo T2 relaxation time. Both models use the concept of a spherical dephasing volume around a spherical object, which generates the magnetic field inhomogeneity. The implications of this model are discussed in detail in [7]. The parameter range of the intracellular iron uptake was chosen to cover the range of 0.05 – 5 pg Fe/cell and the cell count was investigated in the range of 10^4 up to 10^7 cells/ml.

Results and Discussion

An investigation of both models shows the contour lines have only one point of intersection in the parameter range of interest for both a pair of T2 and T2* relaxation times. This is also valid in the case of a pair of T2 relaxation times with different inter echo time (cf. Fig. 2 and 3). The relative difference of the contour in both models strongly depends on the assumptions of the iron distribution inside the cells. This distribution is modeled by the size of the inner sphere. Thus, in principle both of the theoretical models support the possibility to determine the cell density *in vivo* without prior knowledge of the amount of intracellular iron uptake. This should be possible by investigating both T2 and T2*, or by using the dependence of the T2 on the inter-echo time in multi spin echo sequences. The latter seems preferable since the T2 measurements do not suffer from background gradients. Figures 2 and 3 also show a correct determination of the relaxation times is essential. The sensitivity of T2 measurements, however, is dramatically decreased by internalization of the iron oxide in cells [1]. Thus, the method of choice must be adapted on the actual experimental conditions. One problem of the present models is the simplification that internalized iron oxide is considered to agglomerate in a single sphere. Rad et al. [4] could recently show differences in the T2 relaxation time within different cell types despite the same intracellular iron amount. This strongly suggests an influence of the iron oxide distribution inside the cell and should be further investigated.

Acknowledgements

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References

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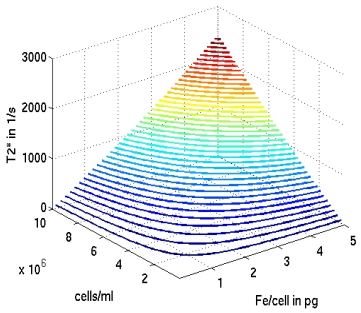

Figure 1: T2* relaxation time dependent on the cell density and iron uptake per cell, calculated with the model from Ziener [5]. As could be clearly seen, for a given T2* value (corresponding to a contour line), a unique determination of the cell density requires knowledge of the iron uptake per cell.

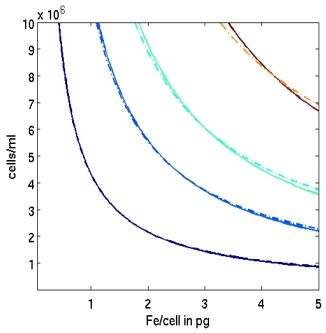

Figure 2: Contours of T2* relaxation time (solid) and T2 relaxation time (dashed) dependent on the cell density and iron uptake per cell, calculated with the model from Ziener [5]. Contours corresponding to a single T2 and a single T2* value show only one intersectional point, leading to an exact determination of cell density and iron uptake.

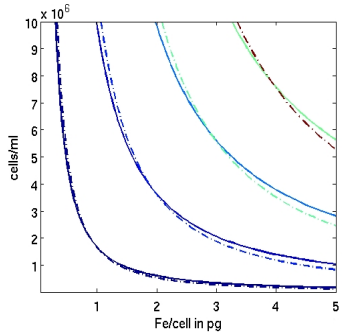

Figure 3: Contours of T2 relaxation times with the interecho times 1 ms (solid) and 20 ms (dashed) dependent on cell density and iron uptake per cell, calculated with the model from Jensen [6]. Contours corresponding to one pair of T2 values show only one intersectional point, leading to an exact determination of cell density and iron uptake.