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Effect of BSA Protein on the Contrast Properties of Magnetite Nanoparticles during MRI

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The aim of the study was to establish whether there is a significant change in the MRI contrast of magnetite nanoparticles, after BSA protein binding on the surface of particles. The rationale is the applicability of this feature in clinical practice for the tracking of specific proteins which are often associated with various pathologies. Contrast agents could bind to this specific marker, with the change in MRI contrast indicating the presence of pathology. We found that changes in relative contrast acquired at low-field MRI offer potential for the differentiation of magnetite nanoparticles with and without BSA protein. However, the variations in the transverse relaxation time (T_2) and transverse relaxivity (r_2), acquired at high-field MRI, were too small to be applicable for biomedical applications.

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1. Introduction

Currently, magnetic nanoparticles in combination with proteins are attracting significant interest in biomedical applications such as specific protein MRI contrast agents [1]. Changes in the concentration levels of proteins are associated with various pathological processes, and as such they can function as biomarkers of various diseases (e.g. cancer [2], neuroinflammation [3]). Magnetic nanoparticles, as a consequence of proton spins coupling with larger magnetic moments of nanoparticles, reduce the transverse relaxation time (T_2), thus increasing the relaxivity of water [4]. Protein binding to the nanoparticle can potentially affect the coupling mechanism, changing the MRI signal and providing desired information regarding the presence of a biomarker. Therefore, the aim of this study was to determine the degree of the BSA protein's influence on the relative contrast and relaxivity of the magnetite nanoparticles stabilised by PEG, after binding to the particles. Quantification of such influence could facilitate the diagnostics of disorders associated with the presence of specific proteins.

2. Materials and methods

PEG-stabilised magnetite nanoparticles were prepared by the co-precipitation method of ferric and ferrous salts in an alkaline aqueous medium, as described in [5]. Particles were made with and without (control) the BSA protein, and then diluted in such a way that each of the samples had half the concentration of the previous one, resulting in a concentration gradient from 3.8 mg/ml to 9.06×10^{-7} mg/ml.

MRI measurements were performed at two types of magnetic fields:
(i) Clinical 0.2 T system ESAOTE — images were acquired with standard T_2-weighted spin echo (SE) pulse sequence, repetition time $TR = 1500$ ms, echo time $TE = 50$ ms.
(ii) Experimental 4.7 T system VARIAN — T_2 was obtained spectroscopically by Car–Purcell–Meiboom–Gill (CPMG) echo pulse sequence.

For all the acquired samples the relative contrast (RC), transverse relaxivity (r_2) and T_2 values were evaluated and compared.

The relative contrast is defined as follows:

$$RC = (I - I_0)/I_0, \tag{1}$$

where I_0 is the signal intensity without magnetite nanoparticles, and I represents the signal intensity with magnetite nanoparticles.

The r_2 is calculated through

$$R_2 = r_2 C + R_2^0, \tag{2}$$

where R_2^0 is the transverse relaxation rate in the absence of nanoparticles, R_2 represents the transverse relaxation rate in the presence of nanoparticles, and C is the nanoparticles' concentration.

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The differences in the relative contrast (RC_{diff}) of the same samples, with and without BSA protein, were then evaluated as follows:

$$RC_{\mathrm{diff}} = |RC_{\mathrm{BSA}(-)} - RC_{\mathrm{BSA}(+)}|, \quad (3)$$

where $RC_{\mathrm{BSA}(-)}$ is the relative contrast of the magnetite nanoparticles without BSA protein, and $RC_{\mathrm{BSA}(+)}$ is the relative contrast of the magnetite nanoparticles with bound BSA protein.

The same process was also carried out for the T_2 in order to evaluate the $T_{2\mathrm{diff}}$.

3. Results

Figure 1 presents the relative contrast of magnetite nanoparticles with (green) and without (blue) BSA protein binding acquired at low-field system. Although not identical, the shape of the curves is very similar. It is obvious that in both cases the optimum contrast change interval lies between samples 6 and 11, which corresponds to the magnetite concentration of $3.71 \div 59.375$ μg/ml. For example, the recommended concentration of the MRI contrast agent Resovist is ≈ 97 μg/ml. The higher concentrations (samples 1–5) were due to vast hypointensive artefacts being utterly undifferentiable. To the contrary of the negative contrast influence of iron oxide nanoparticles in the T_2-weighted MRI images, we observed an increase in the relative contrast value for samples 11–17. This is likely to be caused by the prevailing longitudinal relaxation mechanism in lower particle concentrations. The magnetite concentration of 3.71 μg/ml apparently represents the critical point, where the transversal relaxation mechanism begins to dominate the longitudinal relaxation.

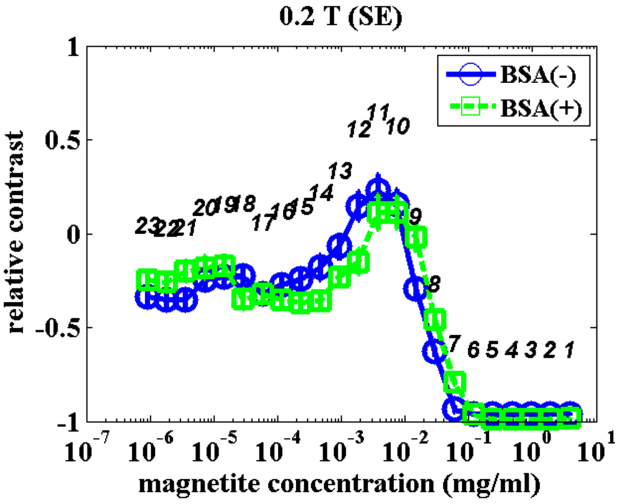

Fig. 1. Relative contrast of magnetite nanoparticles with (green line) and without (blue line) BSA protein binding, in comparison with magnetite concentration (logarithmic scale). Black ciphers represent the sample number. Data were acquired at 0.2 T with T_2-weighted SE pulse sequence.

The quantitative changes (RC_{diff}) in the relative contrast of magnetite nanoparticles with and without the BSA protein in low-field system are shown in Fig. 2.

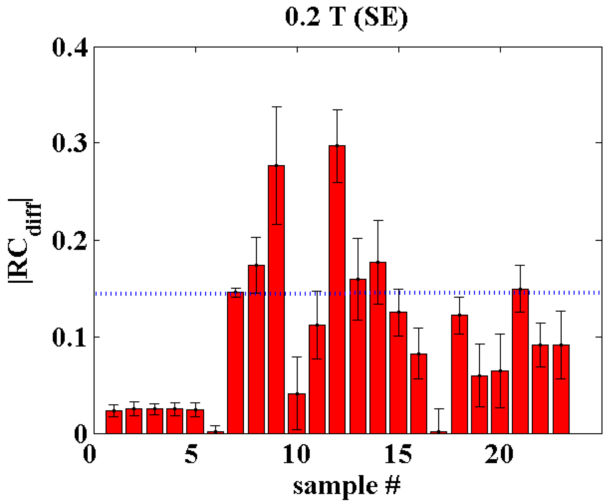

Fig. 2. Difference in relative contrast of magnetite nanoparticles with and without BSA protein binding, in comparison with magnetite concentration (sample number). Data were acquired at 0.2 T with T_2-weighted SE pulse sequence. Blue line represents the contrast change visible to the naked eye.

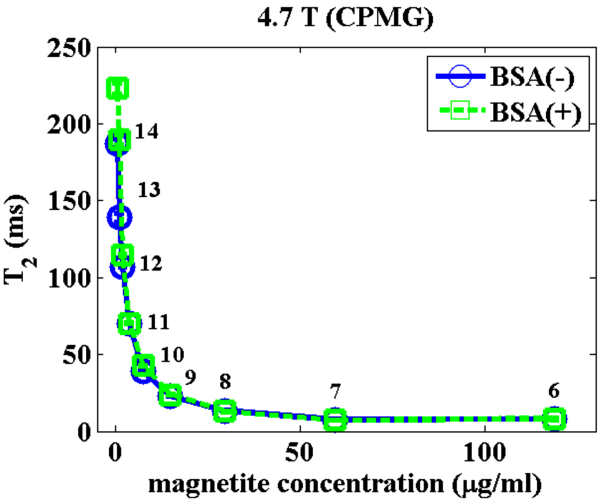

Fig. 3. T_2 of magnetite nanoparticles with (green line) and without (blue line) BSA protein binding, in comparison with magnetite concentration. Black ciphers represent the sample number. Data were acquired at 4.7 T with CPMG pulse sequence.

The blue line represents the contrast change visible to the naked eye in the low-field system ($\approx$ 15%). Five samples exceed this border (8, 9, 12, 13, 14), although only two of them cross the 20% (9, 12) threshold.

These results suggest that the relative contrast comparison of samples with and without protein binding is theoretically possible at low-field MRI, but only for magnetite concentrations ranging from 4 to 60 μg/ml (samples 11 and 7). This is due to the higher concentration levels forming the strong hypointensive artefacts, which disrupt the entire signal. On the other hand, in lower concentration levels we observed a prevailing longitudinal relaxation mechanism, which is not desired in T_2-weighted imaging.

Based on the results at low-field MRI, we selected samples 6–14 for relaxivity measurements at high-field MRI. In Fig. 3 the T_2 is shown, acquired at 4.7 T. We observed almost identical curve shapes for the samples with and without BSA protein, with maximal change in T_2 (14%) for concentrations around 1 μg/ml (Fig. 4). The results indicate that protein binding has only a faint effect on the T_2, when measured at high-field system. This is contrary to the relative contrast comparison at low-field MRI.

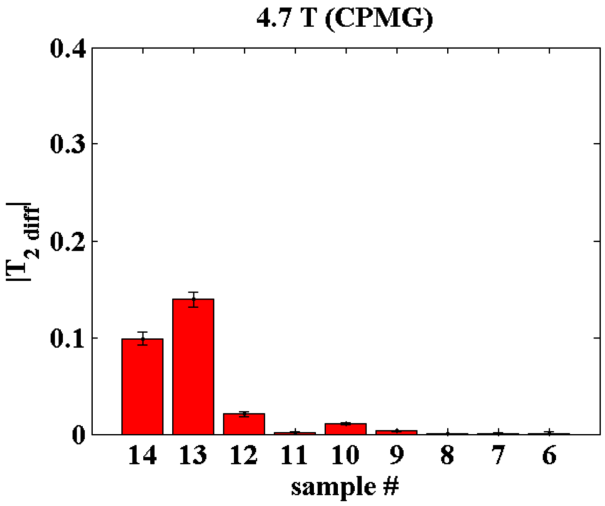

Fig. 4. Difference in T_2 of magnetite nanoparticles with and without BSA protein binding, in comparison with magnetite concentration (sample number). Data were acquired at 4.7 T with CPMG pulse sequence.

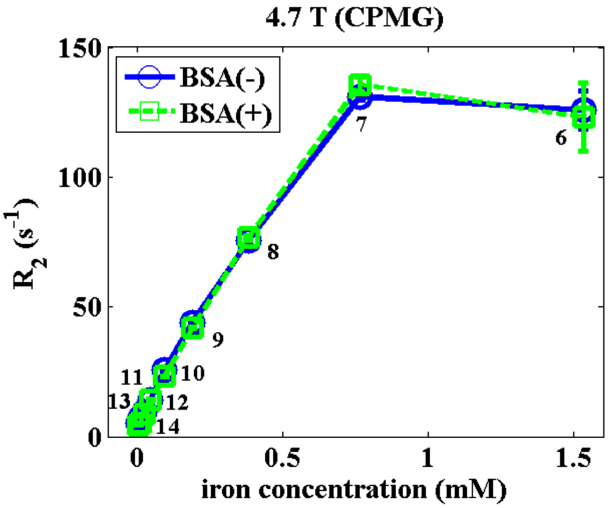

Fig. 5. Transverse relaxation rate (R_2) of magnetite nanoparticles with (green line) and without (blue line) BSA protein binding, in comparison with magnetite concentration. Data were acquired at 4.7 T with CPMG pulse sequence.

The same concentration upper limit (60 μg/ml, sample 7) seen in the relative contrast comparison was also found in the transverse relaxation rate (Fig. 5). Therefore, only samples 7–14 were used for the calculation of the r_2 values. We found the relaxivity of magnetite nanoparticles as follows:

- without BSA protein:
  $r_2^{\mathrm{BSA}(-)} = 165.97 \pm 1.4$ (mM s)$^{-1}$,
- with BSA protein:
  $r_2^{\mathrm{BSA}(+)} = 173.57 \pm 3.2$ (mM s)$^{-1}$.

This agrees with typical r_2 values of small iron oxide nanoparticles — in the order of 100 (mM s)$^{-1}$ [6]. However, the difference between the r_2 of particles with and without BSA protein is too small to be applicable in biomedical applications. On the other hand, we used magnetite nanoparticles without specific adjustments, which could possibly increase the variance.

4. Conclusions

We showed that changes in the relative contrast of magnetite nanoparticles with and without BSA protein, and acquired at 0.2 T, have potential in the differentiation of particles after binding to the protein. This could be helpful in the tracking of specific proteins (markers) associated with various pathologies. However, we did not observe the same variations in T_2 and r_2, acquired at 4.7 T. In this case, the variations were too small to be applicable in clinical practice.

Acknowledgments

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