SPIO-Alginate Nanoparticles: New Platform for Calcium MR Imaging

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Introduction

Ca^{2+} plays a vital role in many biological processes including cell differentiation and apoptosis, cardiac contractility, neurotransmission and brain function. [1] Because of the importance of Ca^{2+} levels in maintaining proper function and in diseases, there is an increasing need for the development of new robust imaging platforms to perform *in vivo* detection of changes in Ca^{2+} levels in wide range of concentrations. Currently, Ca^{2+} imaging is mostly based on fluorescent methodologies.[1] Recently, some progress has been achieved in the design of specific probes for indirect Ca^{2+} MR imaging based on T1 or T2 relaxation.[3-5] Combining the facts that alginates polysaccharides can be cross-linked, selectively, by Ca^{2+} ions[6] and that SPIO aggregation may change the contrast of T2* and T2 WI, we hypothesized that SPIO-alginate nanoparticles may be used as "smart" MRI probes for Ca^{2+} detection (as shown schematically in Fig 1). In this study we describe the synthetic procedure of the preparation of stable water-soluble SPIO-alginate nanoparticles and the ability of these MRI probes to specifically detect different Ca^{2+} concentrations in different media (water, serum and culture cell media) at physiological conditions (pH of 7.2 and 37.0°C).

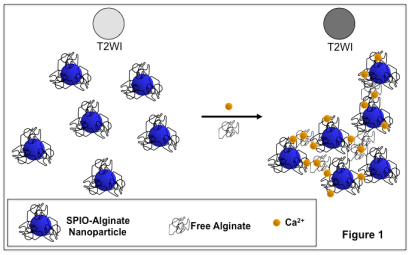

Figure 1

Experimental:

SPIO-alginate nanoparticles were prepared by the coprecipitation methodology. Briefly, 1M NaOH solution was added dropwise to a stirring water solution of Ferric ($FeCl_3$) and Ferrous ($FeSO_4$) salts. Alginate (VLVG, 1% w/w in water) was added to the solution and the reaction flask was heated to 80°C for 1 hour. Then the reaction mixture was sonicated for 20 min and the water soluble SPIO-nanoparticles were obtained after 20 min centrifugation (8500 rpm). Dynamic Light Scattering (DLS): Averaged particles sizes were determined by DLS experiments, which were performed in 10 mM HEPES buffer, pH=7.2. The studied solutions (H_2O for reference/ Ca^{2+} 0.5 mM/ Mg^{2+} 0.5mM) were incubated with SPIO-alginate particles for 3 hours at 37°C before DLS measurements. MRI: Each examined capillary contained SPIO-alginate solution, 10 mM HEPES buffer (pH of 7.2) and sampled solution in a way that the final Ca^{2+}/Mg^{2+} could be determined. MRI experiments were performed using a 8.4T spectrometer (Bruker, Germany). T2WI experiments were acquired using spin echo sequence with 16 echoes (TR=5000 ms and TE=10,20…160 ms). Four mm slice was acquired with a field of view of 1.28×1.28 cm^2 and 256×128 digital resolution reconstructed to a 256×256 matrix. The percentage changes in the MRI signal intensity was calculated as follows: $\%Change=100*(S_s-S_R)/S_R$ where S_s and S_R are the averaged signal intensity of the sample and of the reference, respectively.

Results

We examined our hypothesis by visual inspection (Fig 2a), by DLS (Fig 2b), and by MRI (Figs 2c & 2d). These figures demonstrate the concept of Ca^{2+} MR imaging using SPIO-alginate nanoparticles. Fig 2a shows that 3 hours after the addition of SPIO-alginate solution to the aqueous solutions of M^{2+} (either Ca^{2+} or Mg^{2+}) brown sediments were observed only in the 1.0 mM Ca^{2+} solution. It appears that in the presence of 1.0 mM of Ca^{2+} SPIO-alginate nanoparticles aggregate and precipitate. Fig 2b depicts, by DLS, that aggregation of SPIO-alginate nanoparticles occurs at lower levels of Ca^{2+}. This figure shows that following the addition of the SPIO-alginate nanoparticles to the 0.5 mM M^{2+} solution (M^{2+}=Ca^{2+} or Mg^{2+}), the particles sizes detected by DLS were significantly larger in the Ca^{2+} solution (150±10 nm) compared to the H_2O (reference, 70±10 nm) or Mg^{2+} (80±15 nm) solutions. These differences in the measured dynamic radii demonstrate that 0.5 mM Ca^{2+} results in a soluble SPIO-alginate aggregates. Interestingly, in the presence of Mg^{2+} the observed dynamic radius was very similar to that obtained in the presence of water implying that no significant aggregation occurred with Mg^{2+}. These results are supported by the MR images presented in Fig 2c, which shows that the MRI contrast is influenced by Ca^{2+} level. At Ca^{2+} concentration of 0.5 mM the MR image is darker in spin echo images (TR/TE=5000/20ms) compare to the water containing tube. At higher levels of Ca^{2+}, i.e., 1.0 mM (and more, data not presented) the MR image is brighter comparing to the solutions contained either water or Mg^{2+}. The percentage differences in the MR signal intensity compare to solution without M^{2+} ions are given in Fig 2d showing the sensitivity and the selectivity of SPIO-alginate nanoparticles to Ca^{2+} concentrations. Our findings (Figs 2a-d) indicate that the aggregates sizes as well as aggregation level depend on Ca^{2+} concentrations. At lower Ca^{2+} levels the aggregates are small enough to remain soluble and therefore the T2-weighted MR image is darker compare to non-aggregate particles (in the presence of water or Mg^{2+}). At Ca^{2+} of 1.0 mM the obtained aggregates seems to be bigger and non-soluble (Fig 2a) and therefore the T2WI is brighter as a result of the removal of the SPIO-alginate from the detected solution.

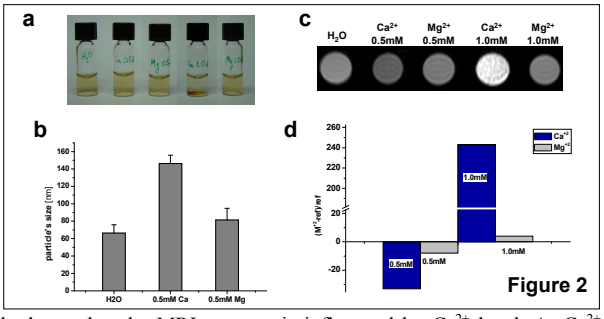

Figure 2

In addition, our Ca^{2+} nanoparticles based sensor shows capability to image different Ca^{2+} concentrations in the presence of Mg^{2+} at physiological levels, i.e., 0.5 mM (Fig 3a) as well as in different physiological media, i.e., fetal bovine serum (FBS, Fig 3b) and Dulbecco's Modified Eagle Medium (DMEM, Fig 3c). Figs 3 a-c depict the sensitivity of our probe to Ca^{2+} levels by plotting the percentage changes in the MRI T2 weighted signal intensity (relatively to Ca^{2+}-deficient sample) of samples with different levels of Ca^{2+}. It is important to note that we can tune our MRI SPIO-alginate probe to be more sensitive to different regimes of Ca^{2+} concentration and in different media.

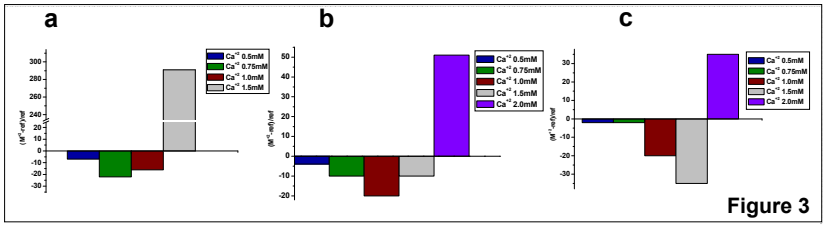

Figure 3

Conclusion:

In this study we demonstrate the capability of SPIO-alginate nanoparticles to serve as MRI biomarker for specific detection of Ca^{2+} levels in different physiological solutions. These stable magnetic nanoparticle probes, which can be rapidly obtained in ample quantities, enables MR imaging of Ca^{2+} levels in different concentration regimes which may be relevant to different biological processes. In addition to their specificity to Ca^{2+} ions as compared to Mg^{2+}, these probes have the ability to image normal as well as abnormal calcium concentrations even in the presence of magnesium and at different biological media such as FBS and DMEM. The versatility, flexibility as well as the robust results obtained with these SPIO-alginate probes for Ca^{2+} concentration may provide a means to non-invasively detect normal and abnormal Ca^{2+} concentrations in-vivo which may increase our ability to detect different diseases and better understand the role of Ca^{2+} in different pathologies.

References: [1] Hofer AM & Brown EM. Nat. Rev. Mol. Cell Biol. 2003;4:530-538. [2] Rochefort NL et al. Trends in Mol. Med. 2008;14:389-399. [3] Li W et al. J. Am. Chem. Soc. 1999;121:1413-1414. [4] Dhingra K et al. Chem. Comm. 2008;29:3444-3446. [5] Atanasijevic T et al. Proc. Nat. Acad. Sci. U.S.A. 2006;103:14707-14712. [6] Fang Y et al. J. Phys. Chem. B 2007;111:2456-2462.