INTRODUCTION AND PURPOSE

Nanoparticles (NPs) such as iron oxide are potential contrast agents (CA) for magnetic resonance imaging (MRI). The determination of CA characteristics in vitro is the first step for their application in vivo. We measured basic characteristics (relaxivity in three solutions types, in two different strengths of magnetic field and stability in time) of experimental ultrasmall superparamagnetic iron oxide (USPIO, less than 20 nm diameter) NPs. USPIO has a potential for in vivo cell imaging as nonspecific CA.

METHODS

Materials

Maghemite particles were made by chemical coprecipitation method of iron ions in alkaline solution and stabilised with biocompatible citrate shell [1]. USPIO NPs size was estimated by atomic force microscopy as 2-25 nm (NanoWizard 3 BioScience) with a median of 3 nm, which was verified by electron microscopy (Magellan, FEI) to be 2 nm (Fig 1a). We used three solution types prepared by mixing USPIO storage solution (450 mmol/mL) with either demineralised water, saline solution (NaCl 0.9 %, Braun, Germany) or saline solution with added albumin (A7906, Sigma-Aldrich, 0.65 mg albumin in 1 mL). Eight samples of each type were prepared: NP concentrations 45, 22.5, 9, 4.5, 2.25, 0.9, 0.45 and 0 mmol/mL, the last being a reference sample containing no NPs. All samples were aliquoted into 1.5 mL Eppendorf microtubes at 1 mL volume for 9.4 T system. For measurements on 1.5 T system we prepared saline solution with NP concentrations 90, 45, 22.5, 11.3 and 0 mmol/mL, aliquoted into 10 mL syringe.

MRI relaxometry

MRI acquisitions were performed on a 1.5 T (Siemens-Avanto) and a 9.4 T (Bruker-BioSpec 94/30USR) systems. For T1- and T2-measurement we used two-dimensional Rapid Acquisition with Refocused Echoes (RARE) (TR = 167-15 000 ms; TE = 10-150 ms, rare factor = 2), for T2-measurement Multi-Slice Multi-Echo (MSME) (TR = 1 500, 2 500 ms; TE = 11-250 ms) and for T2* Multiple Gradient Echo (MGE) (TR = 2 500 ms, TE = 4-70 ms, FA = 30°) sequences. Slice thickness (SL) = 1 mm, matrix = 256×256, FOV = 6×4 cm, single slice, slice direction = horizontal, single acquisition. We calculated T1, T2 and T2* values in a ROI chosen from a series of images using Bruker Paravision analysis program.

We used Fast Low Angle Shot (FLASH), Gradient Echo (GE) and Turbo Spin Echo (TSE) sequences to measure relaxation times at 1.5 T. For T1-measurement we used FLASH (TR = 27 ms, TE = 2.75 ms, FA = 90°), for T2*-measurement GE (TR = 1 000 ms, TE = 4-50 ms, FA = 25°); for T1- and T2-measurement TSE (TR = 500-10 000 ms, TE = 17-60 ms) SL = 5 mm, matrix = 128×128, FOV = 12×12 cm, 3 slices, slice direction = horizontal, single acquisition.

RESULTS

We used ImageJ (National Institutes of Health) for ROI analysis and MATLAB (MathWorks) for the calculation of relaxation times. The r1, r2 and r2* relaxivity values were determined as proportionality constants of the linear relation between the CA concentration and reciprocal relaxation time.

USPIO NPs were stable in time (Fig 2) and did not coagulate in solutions during one month experiment period (only saline solution with concentration 11.3 mmol/mL for measurement on 1.5 T coagulated after three weeks).

CONCLUSIONS

This type of USPIO NPs could be promising CA for cell imaging due to very small size and good relaxivity ratio r2/r1, but further research on potential toxicity on cells is needed.