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T$_1$-Weighted Ultrashort Echo Time Method for Positive Contrast Imaging of Magnetic Nanoparticles and Cancer Cells Bound With the Targeted Nanoparticles

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Abstract

**Purpose**—To obtain positive contrast based on T$_1$ weighting from magnetic iron oxide nanoparticle (IONP) using ultrashort echo time (UTE) imaging and investigate quantitative relationship between positive contrast and the core size and concentration of IONPs.

**Materials and Methods**—Solutions of IONPs with different core sizes and concentrations were prepared. T$_1$ and T$_2$ relaxation times of IONPs were measured using the inversion recovery turbo spin echo (TSE) and multi-echo spin echo sequences at 3 Tesla. T$_1$-weighted UTE gradient echo and T$_2$-weighted TSE sequences were used to image IONP samples. U87MG glioblastoma cells bound with arginine-glycine-aspartic acid (RGD) peptide and IONP conjugates were scanned using UTE, T$_1$ and T$_2$-weighted sequences.

**Results**—Positive contrast was obtained by UTE imaging from IONPs with different core sizes and concentrations. The relative-contrast-to-water ratio of UTE images was three to four times higher than those of T$_2$-weighted TSE images. The signal intensity increases as the function of the core size and concentration. Positive contrast was also evident in cell samples bound with RGD-IONPs.

**Conclusion**—UTE imaging allows for imaging of IONPs and IONP bound tumor cells with positive contrast and provides contrast enhancement and potential quantification of IONPs in molecular imaging applications.

**Keywords**
magnetic nanoparticle; magnetic resonance imaging; iron oxide; ultrashort TE; molecular Imaging

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Varieties of magnetic nanoparticles have been introduced as contrast agents for MRI and molecular imaging probes because of their superb ability in shortening transverse relaxation times $T_2$ and $T_2^*$, which leads to a strong decrease in signal intensity of target organs or so-called “negative contrast” on conventional $T_2$-weighted images (1,2). However, the typical drawback of the negative contrast is its poor contrast when used to study areas that have low background signals. It is challenging to image magnetic nanoparticle labeled cells or magnetic nanoparticle based molecular imaging probes with negative contrast, which accumulate in the organs and tissues in the area with high magnetic inhomogeneity or susceptibility effect, e.g., bone marrow and lung, due to the intrinsic tissue signal void. Because of the magnetic susceptibility induced by the strong dephase effect of magnetic nanoparticles, $T_2$ and $T_2^*$-weighted imaging methods are also vulnerable to the “blooming” artifacts and partial volume effects (3,4), making accurate localization and quantitative imaging difficult. Furthermore, higher magnetic susceptibility and stronger $T_2$ effect from large sized magnetic nanoparticles may not be translated to better negative contrast in the $T_2$-weighted imaging, as the contrast may not improve further as the level of signal decrease cannot get beyond voiding the signal from its original level (5).

To overcome these limitations, there are significant efforts in developing alternative imaging methods that are capable of enhancing the signal or generating bright and positive contrast (6–10). For example, several off-resonance imaging techniques have been investigated to image the off-resonant spins of the tissue due to the influence of magnetic nanoparticles to generate positive contrast (9,10). On the other hand, it is known that magnetic nanoparticles also exhibit a higher longitudinal relaxivity, providing intrinsic signal enhancement on $T_1$-weighted images (11–13). Conventional $T_1$-weighted sequences have been tested for this positive contrast mechanism in magnetic nanoparticles based MR angiography (14). However, conventional imaging sequences cannot fully take advantage of such strong $T_1$-weighted contrast effect because of the predominant $T_2$ and spin dephase effects of magnetic nanoparticles.

Ultrashort echo time (UTE) imaging is capable of imaging materials with extremely short $T_2$ and very fast signal decay (15,16). With very short echo time (TE), typically below 0.1 ms, UTE imaging allows for signal acquisition with little $T_2$ influence. In this study, a UTE imaging sequence was applied to obtain $T_1$-weighted positive contrast enhancement from magnetic iron oxide nanoparticles (IONPs) with high $r_1$ and $r_2$ relaxivities. Signal enhancement obtained from the UTE imaging was found to be a function of the $T_1$ relaxation times of the IONPs at different core sizes and concentrations. In addition, the relative-contrast-to-water (RCW) ratios were calculated for UTE images and compared with those of $T_2$-weighted images. The UTE imaging was further tested for the positive contrast imaging of U87MG human glioblastoma cells targeted with an IONP conjugated with a small peptide ligand of arginine-glycine-aspartic acid (RGD), which has a high affinity to the cells overexpressing $\alpha_v \beta_3$ integrin.

**MATERIALS AND METHODS**

**Preparation of Samples**

**Magnetic Iron Oxide Nanoparticles**—IONPs with four different core sizes were prepared and coated with amphiphilic triblock copolymers as previously reported (17). Briefly, hydrophobic iron oxide nanocrystals were prepared by heating iron oxide powder and oleic acid in octadecene over 315°C. This process allows for obtaining small and size-uniformed nanoparticles. The size of IONPs was tuned through modulating experimental conditions such as heating time, temperature, and ratio of the iron oxide and oleic acid. Oleic acids coated on the of IONP surface were replaced by amphiphilic copolymers.
through the ligand-exchange process. Resulting IONPs were transferred and stabilized in water. IONPs in the size ranges used in this study exhibit mostly a spherical morphology as shown in transmission electron microscopy (TEM) images (H-7500, Hitachi, Tokyo, Japan; Fig. 1). The averaged IONP core sizes were obtained by measuring 50 nanoparticles randomly chosen from the corresponding TEM images. IONP colloidal solutions at five different concentration levels for each size were prepared from the IONP stock solutions in which iron concentrations were determined by chemical analysis using a ultraviolet-visible spectroscopic method (DU530 Spectrophotometer, Beckman Coulter, Fullerton, CA). The characteristics of all IONP samples used in this study are summarized in Table 1.

**Cell Samples**—U87MG human glioblastoma cells were seeded in an eight-well chamber slide and then incubated in the serum-free RPMI medium (HyClone, Thermo Scientific, Logan, UT) with a 0.1 mM concentration of IONP (13-nm core size) conjugated with a 9-amino acids peptide containing an RGD unit targeting the integrin $\alpha_v\beta_3$ overexpressed in U87MG human glioma cells for 2 h at room temperature. Cells were then washed twice with the PBS buffer solution and fixed with 4% paraformalin for 20 min. Binding of the integrin $\alpha_v\beta_3$ targeting RGD-IONP conjugates to U87MG cells was confirmed using Prussian blue staining for iron. Each well was given 0.2 mL of a fresh mixture of 4% potassium ferrocyanide (II) trihydrate and 4% HCl solution, and the cells were incubated for 15 min, after which each well was washed two times with water. They were then stained with Neutral Red for 5 min and then rinsed twice with water. Each cover slip was placed on a slide and treated with Permount gel and then dried overnight. The result of Prussian blue staining was assessed by a light microscope. To make cell samples for MRI experiments, RGD-IONP bound cells were washed with the PBS buffer solution and then scraped from the flasks. Collected cells were resuspended in 1 mL of 2% agarose gel at 50°C in a glass tube before the cell mixture cooled to room temperature and solidified.

**MR Imaging**

All MRI experiments were performed on a 3 Tesla (T) MR scanner (Magnetom Tim Trio, Siemens Medical Solutions, Erlangen, Germany) using a standard head coil. The cell containing glass tubes then were mounted in the sample holder and placed in the iso-center of the magnet for MRI scans.

**Measurement of $T_1$ and $T_2$ Relaxation Times**—To measure the longitudinal relaxation time $T_1$ of each sample, an inversion recovery turbo spin echo (TSE) sequence with echo train length (ETL) of 3, echo time (TE) of 8.5 ms and repetition time (TR) of 1500 ms was used to obtain images at different inversion times (TI) of 23, 46, 92, 184, 368, 650, 850, 1100, and 1400 ms, respectively. To measure the transverse relaxation time $T_2$ of each sample, a multi-echo spin echo (SE) sequence was used with TR of 2000 ms and 20 TEs starting at 10 ms with increments of 10 ms.

MR images were analyzed using a MATLAB program (Mathworks, Natick, MA). The signal intensities from selected regions of interest (ROIs) were measured at different TI or TE times. Briefly, circular ROIs were placed on the individual images of each sample, averaging 2.0 cm$^2$ per ROI. Signal intensities of three ROIs selected from three slices of each sample were averaged. The value of the $T_1$ relaxation time was calculated from the averaged signal intensity values $I$ at different TIs by performing a nonlinear curve fitting of the Levenberg-Marquardt algorithm based on Eq. \[1\]:

$$I = K_1 \left( 1 - 2e^{-TI/T_1} + e^{-TR/T_1} \right) \quad \text{[1]}$$

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where $K_1$ is the signal intensity in the absence of $T_1$ weighting for each ROI in this equation. Similarly, the value of the $T_2$ relaxation time was calculated from the measured average signal intensity values $I$ at different $T_E$s using a log-linear least-square curve fitting based on Eq. [2]:

$$I = K_2 e^{-T_E/T_2} \quad [2]$$

where $K_2$ is the signal intensity in the absence of $T_2$ weighting for each ROI in this equation.

The resulting values of $T_1$ and $T_2$ relaxation times with units of seconds were then converted to relaxation rates of $R_1$ ($R_1 = 1/T_1$, s$^{-1}$) and $R_2$ ($R_2 = 1/T_2$, s$^{-1}$). The $R_1$ and $R_2$ relaxation rates of IONPs were plotted as a function of iron concentrations, and then fitted using a linear least-square fitting algorithm implemented in SigmaPlot (Jandel Scientific, Corte Madera, CA). Lastly, longitudinal and transverse relaxivities, i.e., $r_1$ and $r_2$, of the nanoparticles at different sizes were derived from the $R_1$ and $R_2$ relaxation rates, because $r_1$ and $r_2$ relaxivities corresponded to the slopes of the plots of $R_1$ and $R_2$ at different concentrations, respectively.

**UTE Imaging for Contrast Enhancement**—A UTE gradient-recalled echo (GRE) sequence described previously (18) was used to image all the samples. This sequence consists of one 60-µs-long nonselective radiofrequency (RF) pulse followed by a 40-µs transmit/receive switch time, and a 100% asymmetric data acquisition from the center to the surface of a sphere in the $κ$-space using a three-dimensional (3D) radial sampling trajectory. To achieve the shortest possible $T_E$, data acquisition starts during ramp-up time of the readout gradient. The reconstruction program consists of a Kaiser-Bessel gridding algorithm (window width = 3 and $β$ = 4.2054) with sampling density compensation modified to correct for undersampling. No special off-resonance correction was performed. The ultra-short $T_E$ used for this study was 0.07 ms. Other parameters included TR = 21.7 ms, flip angle = 15°, field of view (FOV) = 22 × 22 × 22 cm$^3$, image matrix = 192 × 192 × 192, voxel size = 1.1 × 1.1 × 1.1 mm$^3$, number of readout samples in one radial projection = 384, duration of one radial projection = 883.2 µs, total radial projections = 64000, and total scan time = 1391 s.

For comparison, moderately $T_2$-weighted TSE imaging was performed on IONP samples. Imaging parameters included ETL = 3, TR = 1000 ms, TE = 19 ms, image matrix = 320 × 320, pixel size = 0.7 × 0.7 mm$^2$, slice thickness = 1 mm, and total scan time = 111 s. The selection of moderate $T_2$ weighting instead of a typical strong $T_2$ weighting was due to the consideration of visualizing negative contrast for all IONPs in this study, some of which have extremely short $T_2$ relaxation times.

For cell samples, a dual echo TSE imaging with one TE of 7 ms for moderately $T_1$-weighted contrast and the other TE of 40 ms for moderately $T_2$-weighted contrast was performed with imaging parameters including ETL = 3, TR = 1000 ms, image matrix = 256 × 256, pixel size = 0.16 × 0.16 mm$^2$, slice thickness = 1 mm, and total scan time = 186 s.

**Signal Simulations and Contrast Analyses**

To better understand the overall effect of imaging parameters such as TR, TE, and flip angle on the signal intensity of conventional $T_1$, $T_2$-weighted SE/TSE imaging, and UTE imaging under the influence of different IONPs, a simplified simulation was performed. Specifically, the signal intensity of MR images of the basic GRE sequence at a given TE and TR was simulated using the Eq. [3]:

$$I = K_3 θ e^{-T_E/T_2} \sinθ(1 - e^{-TR/T_1})/(1 - e^{-TR/T_1} \cosθ) \quad [3]$$
where $K_3$ is a constant for this equation, $\rho$ is the proton density, and $\theta$ is the flip angle of the excitation RF pulse. The signal intensity of MR images of the basic SE/TSE sequences was simulated using Eq. [4]:

$$I = K_3 \rho \cdot e^{-TE/T_1} (1 - e^{-TR/T_1})$$ [4]

where $K_3$ is a constant for this equation. The arguments of TR, TE and $\theta$ were the corresponding experimental parameters, $T_1$ and $T_2$ were experimentally determined as described above, while $K_3$, $K_4$ and $\theta$ can be neglected. All of these argument values were then substituted into Eqs. [3] and [4] to calculate the theoretical signal intensities at each condition. In practice, as a reasonable estimation, $T_2$ was substituted for $T_1$ in Eq. [3] when simulating the signal intensity of MR images of the basic GRE sequence because the $T_2^*$ values of IONP samples could not be accurately measured by conventional MR sequences due to their strong signal void on the image, especially with the large sized IONPs.

To compare the contrast enhancing effects of the $T_1$-weighted UTE and conventional $T_2$-weighted TSE methods, a simple contrast analysis was performed using the signal intensity of pure water as a reference. After measuring signal intensities of each IONP sample and water, RCW ratios of $T_1$-weighted UTE images and $T_2$-weighted TSE images for each IONP sample were computed using Eq. [5]:

$$RCW = \frac{I_{\text{exp}} - I_{\text{water}}}{I_{\text{water}}}$$ [5]

where $I_{\text{exp}}$ and $I_{\text{water}}$ are the signal intensity of the selected IONP sample and water, respectively.

RESULTS

**$T_1$ and $T_2$ Relaxation Times**

The $R_1$ and $R_2$ values of IONPs at selected iron concentration levels were depicted as a function of the particle core size in Figure 2a,b, and the $R_1$ and $R_2$ values of IONPs at different core sizes were plotted as a function of the iron concentration at the levels of 1–4 in Figure 2c and 2d, respectively. However, results from level 5 were not included in Figure 2 due to the measurements of $T_1$ and $T_2$ with conventional MR imaging sequences were not accurate enough at the high iron concentration. To evaluate the correlations of relaxation times and IONP sizes and concentrations, a general linear regression by linear least-square fitting was performed. Generally good linear correlations between $R_1$ and $R_2$ and the core sizes of IONPs were observed (Fig. 2a,b) with exception of IONPs in the level 1 from which a low linear correlation coefficient between $R_1$ and core sizes were observed possibly due to the relatively large error of the $T_1$ measurement for IONP with very small core size (5 nm) and low concentration (Fig. 2a). Also, good linear correlations between iron concentrations and $R_1$ and $R_2$ values of IONPS at the different core sizes were observed (Fig. 2c,d). The $r_1$, $r_2$ relaxivities and ratios of $r_1$ to $r_2$ of each IONP sample were calculated and listed in Table 2.

**Simulations of Signal Profiles of Different IONPs With Different Imaging Sequences**

The simulated evolution of signal intensities from the UTE, $T_1$ and $T_2$-weighted imaging at different IONP concentrations for different core sizes were plotted in Figure 3. For the typical $T_1$-weighted SE/TSE sequence, the signal intensity of each IONP sample reached a peak value when the iron concentration increased, then dropped (dash-dot line in Fig. 3a) as a result of the dominant $T_2$ shortening effect at a higher iron concentration. In addition, the smaller the size of the nanoparticle was, the broader the concentration range of the $T_1$
enhancement was (Fig. 3a). For the T<sub>2</sub>-weighted SE/TSE sequence, the magnitude of signal intensity increase at low concentrations was smaller than that of T<sub>1</sub>-weighted SE/TSE sequence, because the signal enhancement caused by the T<sub>1</sub> effect was larger than the signal drop caused by the T<sub>2</sub> effect. Similarly, the signal drop was more pronounced for IONPs with larger sizes due to their dominating T<sub>2</sub> effect (Fig. 3b). In contrast, for the T<sub>1</sub>-weighted UTE imaging of the same samples, the increase of signal intensities and the positive contrast enhancement were observed in the entire concentration range and continued to rise as the iron concentration increased (Fig. 3c).

**Contrast Enhancement of IONP with UTE Imaging**

Positive contrast enhancement was obtained in all the IONP samples in this study with the UTE sequence (Fig. 4a). The blurred appearance of the UTE images may be caused by the resonance frequency shift introduced by IONPs (19). In contrast, negative contrast or signal drop, and eventually signal void were observed with the conventional T<sub>2</sub>-weighted imaging (Fig. 4b). Furthermore, the signal intensity increased in T<sub>1</sub>-weighted UTE images as the core size of IONPs increased (Fig. 5a). Similarly, we observed the increased signal intensity and higher positive contrast enhancement of IONPs at different core sizes in UTE images as the iron concentration increased (Fig. 5b).

**RCWs of UTE and T2 Weighted Images**

We compared the efficiency of the contrast enhancement from the T<sub>1</sub>-weighted UTE imaging and the moderately T<sub>2</sub>-weighted TSE imaging using the RCW analysis of images from IONP samples for different core sizes and concentrations using the water signal intensity as the baseline reference. The results of RCW analysis of the different samples are summarized in Table 3. The RCWs of UTE images were three to four times higher than those of T<sub>2</sub>-weighted TSE images from IONP samples of different core sizes and iron concentrations, except the IONP with the core size of 4.8 nm.

**UTE Imaging of Cancer Cells Bound with Targeted IONPs**

IONPs conjugated with the small peptide ligand containing the tumor α<sub>v</sub>β<sub>3</sub> integrin targeting RGD unit exhibited strong affinity to U87MG glioblastoma cells as confirmed by Prussian blue iron staining of U87MG cells treated with RGD-IONP conjugates (Fig. 6a). U87MG glioblastoma cells bound with RGD-IONP conjugates exhibited positive contrast enhancement in UTE imaging as shown in Figure 6b (the second column to the fifth column in the third row) when compared with the control cell sample without the binding of RGD-IONP (the first column in the third row of Fig. 6b). In contrast, the T<sub>1</sub>-weighted TSE images did not exhibit pronounced positive contrast increase in the samples with higher numbers of RGD-IONP conjugates bound cells (the first row in Fig. 6b). The same U87MG glioblastoma cell samples bound with RGD-IONP conjugates showed signal drop and negative contrast in T<sub>2</sub>-weighted imaging (the second row in Fig. 6b). Furthermore, the higher the number of RGD-IONP conjugates bound cells in the sample, the higher the signal intensity observed in UTE images. The level of the contrast enhancement from the different number of IONP bound cells in UTE imaging is consistent with the signal reductions in the T<sub>2</sub>-weighted images of those samples.

**DISCUSSION**

Magnetic nanoparticles have been widely used as MRI contrast agents and molecular imaging probes in clinical and preclinical studies, including MR venography, magnetic cell-labeling for in vivo cell tracking with MRI, biomarker targeted MR molecular imaging and MRI monitored drug delivery (13,14,20,21). Most applications with magnetic nanoparticles as contrast agents are based on the dominant T<sub>2</sub> effect, while the strong T<sub>1</sub> contrast
enhancement effect of magnetic nanoparticles has rarely been used in clinical and molecular imaging. The increase of the $R_1$ relaxation rate at increased particle size and iron concentration of nanoparticles is typical and is due to the increased interactions between the magnetic nanoparticles and surrounding water molecules. Our results were consistent with previous findings that relaxation of signals is dominated by $T_1$ effects at short TE and lower concentration of nanoparticles (22). UTE imaging captures the $T_1$ effect of the magnetic nanoparticles contrast agents that enable obtaining signal enhancing positive contrast complementary to the typical signal voiding “negative” contrast from the $T_2$ effect. Positive contrast enhancement from the increase of the signal intensity in UTE imaging is attributed mainly to the increased $R_1$ relaxation rate of the nanoparticles. To capture the $T_1$ enhancement effect before the rapid signal drop caused by $T_2$ effect from the magnetic nanoparticles, UTE imaging uses rapid transmit/receive switching, radial mapping of $\kappa$ space, and a very short nonselective RF excitation of 60 $\mu$s that allows the signal acquisition to start almost immediately after RF excitation (18). Our study showed that by using a UTE pulse sequence with TE of 0.07 ms, which is significantly shorter than those available on the conventional clinical MRI systems, it is possible to detect signals and obtain $T_1$-weighted positive contrast from the short $T_2$ materials with $T_2$ or $T_2^*$ relaxation times on the order of only a few hundred microseconds (15,16). With an ultrashort TE, the signal enhancing $T_1$ effect by IONPs leads to the signal increase or positive contrast that is not available from conventional sequences. Therefore, the UTE imaging approach may provide an alternative method for gaining IONP-induced contrast in the tissue or organ when the background signal level is low or when $T_2^*$-weighted gradient echo for detection of IONPs may suffer from IONP-induced susceptibility artifacts.

The positive contrast generated from UTE methods takes advantage of the unique effect of magnetic nanoparticles on relaxation time domain. In comparison, previously reported off-resonance imaging methods use spectrally selective RF pulses to excite and refocus the off-resonance water surrounding the magnetic nanoparticles for positive contrast imaging of surrounding tissue (6–8). In addition, inversion-recovery with on-resonant water suppression (IRON) may be used in conjunction with the spectrally selective RF pulses (4). The major limitation of these techniques is the need of an extra image acquisition to provide background anatomic details. Alternatively, fast low-angle positive contrast steady-state free precession imaging (FLAPS) is able to generate positive contrast from the off-resonant spins while suppressing the on-resonant spins in a flip-angle-dependent manner, and the background suppression is controllable, making co-registration of the bright signal of the magnetic nanoparticles and the anatomic details in the same image (9,10). However, this technique is still limited by the magnetic perturbations around the magnetic nanoparticles.

More importantly, we observed that signal enhancement by IONPs in UTE imaging is a function of the concentration and particle size of the nanoparticles in this study. In addition, we found that RCWs of UTE images are three to four times higher than those of $T_2^*$-weighted imaging of the same IONP samples. A linear correlation was found between the signal intensity and iron concentration of the nanoparticle. The deviations at high iron concentrations and large IONP sizes (Fig. 5a) is because the $T_2$ effect becomes profound and not completely negligible as the core size and concentration of IONPs increase, and signal intensities are influenced by both $T_1$ and $T_2$ effects. One can expect that as the sizes of magnetic nanoparticles are increasing, the $T_2$ influence become too strong to overcome even for the UTE imaging used in this study (TE = 0.07 ms). Further development of UTE sequences to use even shorter TE than 0.07 ms would enable the usage of magnetic nanoparticles in a larger size range.

The observed signal intensity increase in UTE images as a function of the increase of the iron concentration may provide a means to quantify the iron concentrations in the tissue for
various applications, such as quantification for the implanted magnetic nanoparticle labeled cell grafts and estimation of the concentration of magnetic nanoparticles or magnetic nanoparticle carriers in vivo. Liu et al recently investigated $T_2^*$ relaxation time in phantoms and tissues containing a focal area of magnetic iron oxide nanoparticle labeled cells with UTE imaging (22). They proposed that UTE imaging was expected to provide quantification of highly concentrated iron-labeled cells from direct cell transplantation when combined with regular $T_1$ mapping. Our results revealed that the signal intensities of IONP-enhanced $T_1$-weighted UTE images are well correlated to the size and concentration of IONPs in the range used in this study. Therefore, although some similar drawbacks still exist such as the difficulty of separating signals from hemorrhage or endogenous iron particles, our findings demonstrated that UTE imaging may offer a fairly simple approach for quantifying IONPs in vivo as suggested by Liu et al (22), because no extra parameter mapping is needed.

It should be noted that the longitudinal relaxation rate $R_1$ has a greater increase than transverse relaxation rate $R_2$ in IONPs with a core size less than 25 nm and an iron concentration below 1 mM (approximately 0.056 mg Fe/mL) as observed in our study. Such $T_1$-weighted positive contrast is then replaced by increased $T_2$-weighted “negative” contrast as the $R_2$ relaxation time increases sharply at larger core sizes and higher concentrations as predicted in the simulation. This finding suggests that an appropriate IONP core size and concentration range needs to be considered for obtaining positive contrast with UTE imaging. It is considered that the $R_1$ relaxation rate is a function of the surface area that decreases with an increase of nanoparticle size (23) and clustering of the nanoparticles, while the increase of $R_2$ relaxation rate with the increased magnetic nanoparticle concentration is associated to a decrease in the water diffusion coefficient (24). A saturation effect based on the so-called water exchange (25,26) may be present when describing the apparent MRI contrast with IONPs that have the property of increasing both $R_1$ and $R_2$ relaxation rates. In our cases, higher $T_1$ contrast of IONPs with lower concentrations and smaller core sizes may be explained as favorable water diffusion to the IONP and larger surface area with smaller IONPs provide close interactions of the IONP with surrounding water molecules. In comparison, higher $T_2$ contrast from IONPs with higher concentrations and larger core sizes may be due to decrease of the IONP surface area and the clustering of particles which limit the IONP contacting with water molecules.

We also demonstrated the ability to obtain $T_1$-weighted positive contrast from UTE imaging using the receptor targeted IONP molecular imaging probe in vitro. IONPs conjugated with peptide ligands containing a tumor integrin targeted RGD unit have shown strong affinity to human glioblastoma cells with overexpression of integrin $\alpha_v\beta_3$ (27). Integrins are families of cell surface receptors that play important roles in regulating cell adhesion, migration, growth, differentiation, and apoptosis (28). The integrin-binding activity of the RGD containing peptide, which displays a strong affinity and selectivity to the $\alpha_v\beta_3$ integrin, enables the binding of targeting IONPs to U87MG cells that overexpress $\alpha_v\beta_3$ integrins. Our data showed that $T_1$-weighted positive contrast is distinguishable from samples containing $2 \times 10^6$ cells/mL bound with RGD-IONP conjugates, although the approach of $T_2$ relaxometry mapping also showed good sensitivity and strong negative contrast in the imaging of IONPs bound cell samples. It is anticipated that contrast effects of UTE imaging of magnetic nanoparticles could be more complicated in vivo. Further investigations on the effects of surface binding and cellular compartmentalization of magnetic nanoparticles on the contrast of UTE imaging is granted to develop UTE imaging for cellular and molecular imaging with magnetic nanoparticles.

Finally, positive contrast enhancement using UTE imaging may open the opportunity for the applications of magnetic nanoparticles with a strong $T_1$ effect but also extremely short $T_2$. With $T_2$-weighted contrast, the maximum contrast is when the signal drops to zero from its
original level, therefore, once the signal is completely voided, increase of the particle size and concentrations may not further improve the negative contrast. In this circumstance, the advantage of using larger sized IONPs for cell tracking and molecular imaging applications (5) may not be fully appreciated. In contrast, higher positive contrast may be obtained from the larger sized magnetic nanoparticles using T1-weighted UTE imaging, because the R1 relaxation rate is proportional to the nanoparticle size.

In conclusion, positive T1 contrast from magnetic nanoparticles and cancer cells bound with biomarker targeted IONPs can be obtained using UTE imaging, resulting in higher RCWs compared with that of T2-weighted imaging. Increases of the signal intensity and positive contrast of IONPs in UTE images are correlated to the increases of the size and iron concentration of IONPs, allowing for potential quantification of IONPs and IONP-labeled cells in vitro and in vivo.

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Figure 1.
Transmission electron microscopy images of IONP with different averaged core sizes: (a) 4.8 nm, (b) 8.7 nm, (c) 13.4 nm, and (d) 25.4 nm. The scale bar of the images is 50 nm. The core sizes of each type of the IONP were averaged from the measurements of 50 nanoparticles selected from a ROI.
Figure 2.
Plots of relationships between relaxation times and the core sizes or iron concentrations of IONP samples used in this study. 

a: $R_1$ relaxation times vs. the core sizes of selected IONP samples. 
b: $R_2$ relaxation times vs. the core sizes of selected IONP samples. 
c: $R_1$ relaxation times vs. the concentrations of selected IONP samples. 
d: $R_2$ relaxation times vs. the concentrations of selected IONP samples. 
Pearson correlation coefficients of each plot are indicated in the figure.
Figure 3.
Theoretical signal intensity curves of (a) T$_1$-weighted SE/TSE, (b) T$_2$-weighted SE/TSE, and (c) T$_1$-weighted UTE GRE sequences as the functions of the concentration of IONPs with different core sizes. The simulations of the evolutions of signal intensities with different imaging sequences are calculated from experimentally measured relaxation times and IONP core size and concentrations. Signal intensity is in arbitrary unit (marked as A.U. in the figures).
Figure 4.
Comparison of \(T_1\)-weighted UTE and conventional \(T_2\)-weighted TSE images of IONPs. **a:** \(T_1\)-weighted UTE images of selected IONP samples. **b:** Conventional \(T_2\)-weighted TSE images of the same samples. In each image panel, the top row is the IONP samples with a core size of 25.4 nm and the bottom row is the IONP samples with a core size of 8.7 nm, and the concentrations are corresponding to level 1 (left most) to level 5 (right most). UTE images have lower in plane resolution (1.1 \(\times\) 1.1 mm\(^2\) isotropic) than that of \(T_2\)-weighted TSE images (0.7 \(\times\) 0.7 mm\(^2\) isotropic).
Figure 5.
Plots of the signal intensity of T$_1$-weighted UTE images of IONP samples with different core sizes (a) and concentrations (b) show stronger positive contrast with higher iron concentrations and larger core sizes. Pearson correlation coefficients of each plot are indicated in the figure legends. Signal intensity is in arbitrary unit (marked as A.U. in the figures).
Figure 6.
a: Prussian blue staining confirmed the presence of tumor integrin targeted RGD-IONP conjugates in U87MG human glioblastoma cells. b: Comparison of moderately $T_1$-weighted TSE images (top), moderately $T_2$-weighted TSE images (middle) and $T_1$-weighted UTE images (bottom) of cell phantoms containing 0, 1, 2, 4, and $8 \times 10^6$ U87MG glioblastoma cells bound with RGD-IONP conjugates. Cells were embedded in 2% agarose gel.
### Table 1

Iron concentrations of IONP samples of different core sizes

<table>
<thead>
<tr>
<th>Core size (nm)</th>
<th>Level 1</th>
<th>Level 2</th>
<th>Level 3</th>
<th>Level 4</th>
<th>Level 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.8 ± 0.9</td>
<td>0.024</td>
<td>0.119</td>
<td>0.239</td>
<td>0.478</td>
<td>0.956</td>
</tr>
<tr>
<td>8.7 ± 0.7</td>
<td>0.026</td>
<td>0.129</td>
<td>0.258</td>
<td>0.516</td>
<td>1.192</td>
</tr>
<tr>
<td>13.4 ± 1.5</td>
<td>0.020</td>
<td>0.100</td>
<td>0.201</td>
<td>0.401</td>
<td>0.802</td>
</tr>
<tr>
<td>25.4 ± 3.2</td>
<td>0.022</td>
<td>0.112</td>
<td>0.225</td>
<td>0.449</td>
<td>0.898</td>
</tr>
</tbody>
</table>

*Core sizes of IONPs were measured from the particles selected from the picture of TEM.

IONP = iron oxide nanoparticle; TEM = transmission electron microscopy.
### Table 2

Longitudinal and transverse relaxivities of IONP with different core sizes

<table>
<thead>
<tr>
<th>Core size (nm)</th>
<th>$r_1$ (mM$^{-1}$·s$^{-1}$)</th>
<th>$r_2$ (mM$^{-1}$·s$^{-1}$)</th>
<th>$r_1/r_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.8</td>
<td>1.17</td>
<td>5.20</td>
<td>0.2249</td>
</tr>
<tr>
<td>8.7</td>
<td>9.89</td>
<td>182.1</td>
<td>0.0543</td>
</tr>
<tr>
<td>13.4</td>
<td>14.60</td>
<td>318.2</td>
<td>0.0451</td>
</tr>
<tr>
<td>25.4</td>
<td>16.75</td>
<td>335.4</td>
<td>0.0499</td>
</tr>
</tbody>
</table>

IONPs = iron oxide nanoparticles.
Table 3
RCW of UTE and conventional T\textsubscript{2} weighted images of IONPs at different core sizes and concentrations\textsuperscript{*}

<table>
<thead>
<tr>
<th>Size (nm)</th>
<th>Level 1</th>
<th>Level 2</th>
<th>Level 3</th>
<th>Level 4</th>
<th>Level 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.8</td>
<td>0.141 (0.141)</td>
<td>0.285 (0.088)</td>
<td>0.277 (0.067)</td>
<td>0.334 (0.147)</td>
<td>0.607 (0.378)</td>
</tr>
<tr>
<td>8.7</td>
<td>0.218 (0.096)</td>
<td>0.547 (0.217)</td>
<td>0.756 (0.027)</td>
<td>1.285 (0.457)</td>
<td>2.387 (0.878)</td>
</tr>
<tr>
<td>13.4</td>
<td>0.134 (0.028)</td>
<td>0.551 (0.069)</td>
<td>0.858 (0.336)</td>
<td>1.597 (0.775)</td>
<td>3.099 (0.982)</td>
</tr>
<tr>
<td>25.4</td>
<td>0.391 (0.122)</td>
<td>0.763 (0.377)</td>
<td>1.150 (0.665)</td>
<td>2.126 (0.935)</td>
<td>3.478 (0.997)</td>
</tr>
</tbody>
</table>

\textsuperscript{*}RCW values of T\textsubscript{2} weighted images for each sample are listed in parentheses along with those of UTE imaging of the same sample. Levels 1 through 5 correspond to the same concentration levels listed in Table 1.

RCW = relative-contrast-to-water; UTE = ultrashort echo time; IONP = iron oxide nanoparticles.