RESEARCH PAPER

Relevance between MRI longitudinal relaxation rate and gadolinium concentration in Gd^{3+}/GO/alginate nanocomposite

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ABSTRACT

Objective(s): Relevance between magnetic resonance imaging (MRI) relaxation rate and concentration of magnetic nanoparticles determines the capability of a nanomaterial to provide MRI contrast. In the present study, alginate was conjugated to gadolinium/graphene oxide nanocomposite to form gadolinium/graphene oxide/alginate nanocomposite, aiming to investigate its effect on the relevance between MRI longitudinal relaxation rate and paramagnetic gadolinium concentration.

Materials and Methods: The physicochemical properties of the nanocomposite and its effect on the cell culture were investigated. Moreover, MRI longitudinal relaxation rates were determined based on the corresponding exponential curves, and the graph of their relevance with gadolinium concentration was plotted.

Results: The average thickness and sheet size of the nanocomposite were three and 100 nanometers, respectively. The nanocomposite showed high cell viability, even at the relatively high concentration of 75 µg/ml. In addition, a linear correlation was observed between longitudinal relaxation rate and gadolinium concentration.

Conclusion: According to the results, the linearity between gadolinium/graphene oxide/alginate nanocomposite and gadolinium concentration, which revealed a high slope, confirmed the potential of the nanocomposite to significantly improve the positive contrast of MR images.

Keywords: *Gadolinium, Graphene oxide, MRI, Nanocomposite*

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INTRODUCTION

Among various medical imaging modalities, magnetic resonance imaging (MRI) employs non-ionizing radiofrequency pulses to provide valuable structural and anatomical information based on proton relaxation rates. In case of small differences between the proton relaxation rates of the tissues, contrast-enhanced MRI could provide medical images with high sensitivity and accuracy [1].

Gadolinium (Gd) chelates are widely used in clinical MRI as the positive contrast materials with a significant effect on the longitudinal relaxation rate [2]. Changes in the relaxation rates that are normalized to the concentration of the magnetic component of a contrast material are characterized by relaxivity [3]. The proton relaxivity of gadolinium chelates is limited due to their short rotational correlation time [2]. On the other hand, gadolinium nanoparticles have been reported to have higher relaxivity compared to gadolinium chelates, which increases their efficacy as the positive contrast material of MRI [4]. Nanoparticles with high relaxivity enable molecular imaging and the detection of low-concentration targets. Additionally, MR images could be acquired using lower doses of the contrast material [3], which reduces the side effects.

Graphene is a carbon structure in the form of two-dimensional single or multi-layer sheets [5]. Graphene and its oxidized derivative (graphene oxide [GO]) have recently attracted the attention of researchers owing to their low toxicity, large surface area, water solubility, and photothermal

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properties [6, 7]. Therefore, these components are considered to be viable candidates for various biomedical applications, such as cancer photothermal therapy [7], drug delivery, and biomedical imaging [8]. The two-dimensional structures of graphene and graphene oxide layers with thin high surface areas provide possibility of encapsulating of various MRI magnetic materials, such as paramagnetic gadolinium [9] manganese [10], and superparamagnetic iron oxide [11, 12]. Furthermore, graphene oxide has been used along with gadolinium chelates (GO-DTPA-Gd) for the contrast enhancement of human liver hepatocellular carcinoma cells [13], while GO-DOTA-Gd has been applied in stem cell labeling [14].

Due to the toxicity of gadolinium ions, they should be used with coating in order to prevent their toxic effects in MRI applications. To this end, gadolinium chelation with macromolecules is performed on conventional MRI contrast materials. In case of gadolinium nanoparticles, they are often coated with various biocompatible materials [15-18].

Alginate is a linear polysaccharide obtained from algae, which is a non-toxic, biodegradable, and biocompatible material [19, 20]. Alginate hydrogels have various biomedical applications, such as tissue engineering, drug delivery, and wound healing [21].

To date, few studies have been focused on the use of gadolinium/graphene oxide. To the best of knowledge, no research has investigated the correlation between the longitudinal relaxation rate of alginate-coated gadolinium/graphene oxide nanocomposite and gadolinium concentration in MRI.

The present study aimed to investigate the effect of gadolinium/graphene oxide/alginate nanocomposite on the correlation between MRI longitudinal relaxation rate and gadolinium concentration.

MATERIALS AND METHODS

Synthesis of the Gadolinium/Graphene Oxide/ Alginate Nanocomposite

Graphene oxide (GO) was prepared based on the modified Hummers' method using natural flake graphite, sulfuric acid (H_2SO_4), and phosphorus pentoxide (P_2O_5) as the initial materials [22]. The prepared GO was added to the aqueous solution of $GdCl_3.6H_2O$ and NaOH (10 ml), sonicated for 60 minutes, and heated at 110°C for 90 minutes, 140°C for 60 minutes, and 180°C for four hours under argon gas. The prepared Gd^{3+}/GO nanocomposite was separated, washed, and dried. The Gd^{3+}/GO/alginate nanocomposite was prepared using the sonochemical-assisted freeze drying method. In this process, 0.1 gram of Gd^{3+}/GO was dispersed in 25 milliliters of distilled water, sonicated for 20 minutes, added to 30 milliliters of the aqueous solution of sodium alginate, stirred for four hours, and freeze-dried eventually.

Characterization of the Gd^{3+}/GO/Alginate nanocomposite

At this stage, the X-ray diffraction (XRD) patterns were determined using a Siemens D500 diffractometer and Cu kα radiation (λ=1.5418 Å, 2θ=10-30°).

The morphology and size of the nanocomposite was observed using Philips ES 30 KW scanning electron microscope (SEM) and Zeiss LEO 912 Omega transmission electron microscope (TEM) at 140 kV.

Toxicity of the Gd^{3+}/GO/Alginate nanocomposite

MTT assay was performed to investigate the cytotoxicity effects of the nanocomposite on the A549 cell lines. At this stage, the cells were cultured in 96-well plates at the cell density of 8×10^3 cells/well in 200 microliters of the culture medium and preserved at the temperature of 37°C for 24 hours in an atmosphere containing 5% CO_2. Afterwards, the nanocomposite solution infused with various Gd^{3+} concentrations was added to the 96-well plates and incubated for 24 hours. After incubation, the absorbance was measured at the wavelength of 570 nanometers using a standard microplate reader. The MTT assay results were expressed as mean and standard deviation (SD).

MR Imaging of the Gd^{3+}/GO/Alginate nanocomposite

To investigate the contrast-enhanced MRI using the Gd^{3+}/GO/alginate nanocomposite, imaging was conducted at 1.5 T using an MRI scanner (Magnetom Avanto Siemens Healthcare, Germany). The temperature of the scanning room was set at 18°C.

A uniform suspension of the nanocomposite was prepared at various concentrations of gadolinium (0, 0.019, 0.038, 0.059, and 0.081 mM) in test tubes. The tubes were vertically inserted

into a water-filled plastic phantom, placed at the center of the clinical head coil.

The MR images were acquired using spin echo sequence with the fixed echo delay time of nine milliseconds and various repetition times (300, 550, 1,000, 1,800, 2,500, and 3,500 milliseconds). The voxel size was set at 0.6×0.6×5.0 cubic millimeters.

The signal intensity of each sample was measured over the MR image by selecting the region of interest at the center of each sample using the ImageJ software version 1.46, which is an image processing program. Changes in the signal intensity at various repetition times were used for the nonlinear fitting of the longitudinal relaxation time curves. The inverted values of the relaxation times were considered as the longitudinal relaxation rates. In addition, the correlation between the longitudinal relaxation rates and gadolinium concentrations was investigated.

RESULTS AND DISCUSSION

Characterization of the Gd^{3+}/GO/Alginate nanocomposite

Fig 1 depicts the XRD patterns of the nanocomposite. The XRD patterns indicated that the diffraction peaks were in accordance with GO without shifts at 2θ, confirming the stability of GO based on Gd^{3+} ion impregnation and coating.

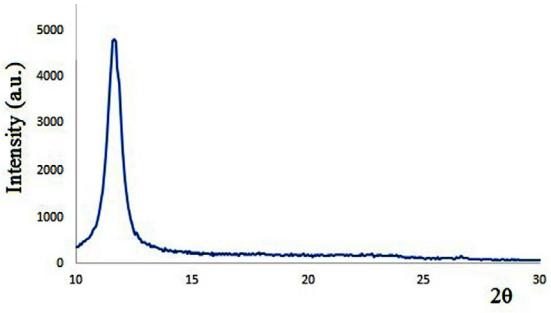

Fig 1. XRD patterns of Gd/GO/alginate nanocomposite

Fig 2-A shows the TEM image of the morphological data of the Gd^{3+}/GO/alginate. As can be seen, the nanocomposite sheets had good separation with the average sheet size of 100 nanometers. The nanoparticles were modified uniformly and firmly onto the surface of GO. The TEM (Fig 2-A) and SEM images (Figs 2-B & 2-C) revealed that the nanocomposite was in a plate-shaped sheet with the thickness of three nanometers, length of 30-130 nanometers, and width of 20-50 nanometers.

Fig 2. Morphology and structure of Gd/GO/alginate nanocomposite characterized by A) TEM, B) and C) SEM

Cytotoxicity of the Gd^{3+}/GO/Alginate nanocomposite

Fig 3 depicts the effect of the Gd^{3+}/GO/alginate nanocomposite on A549 cell viability after incubation for 24 hours. As can be seen, the cells exhibited adequate viability (80%) after incubation with the nanocomposite, even at the relatively high concentration of 75 µg/ml. The results revealed that the prepared nanocomposite was observed to have adequate cytocompatibility.

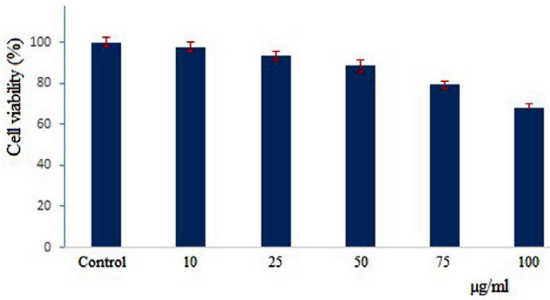

Fig 3. Cell viability of A549 cells exposed to Gd/GO/alginate nanocomposite obtained by MTT assay

MRI study of the Gd^{3+}/GO/Alginate nanocomposite

Fig 4-A shows the MR image of the Gd^{3+}/GO/alginate nanocomposite with various gadolinium ion concentrations. Evidently, the signal intensity of the samples was observed to enhance by increasing the concentration of gadolinium from zero to 0.081 mM. The signal intensity of the highest concentration was 2.3 and 1.2 times higher compared to the concentrations of zero and 0.019, respectively. Therefore, it could be concluded that gadolinium had significant effect on signal changes, which in turn provided contrast.

The longitudinal relaxation curves indicated the exponential increase of signal intensity as a function of the repetition time. As a sample, the longitudinal relaxation curve obtained at the repetition time of 1,000 milliseconds and echo delay time of nine milliseconds is seen in Fig 4-B; inset.

According to the findings, the longitudinal relaxation times were decreased at increased

gadolinium concentrations. In gadolinium-based nanostructures, large numbers of the Gd^{3+} ions interact with water protons, and the interactions increase at higher concentrations of gadolinium. As a result, the longitudinal relaxation time reduces, and signal intensity increases. Furthermore, the high surface area of graphene oxide for the placement of the gadolinium ions could provide more significant interactions between the Gd^{3+} ions and the surrounding water protons, leading to further changes in signal intensity, relaxation time, and relaxation rate.

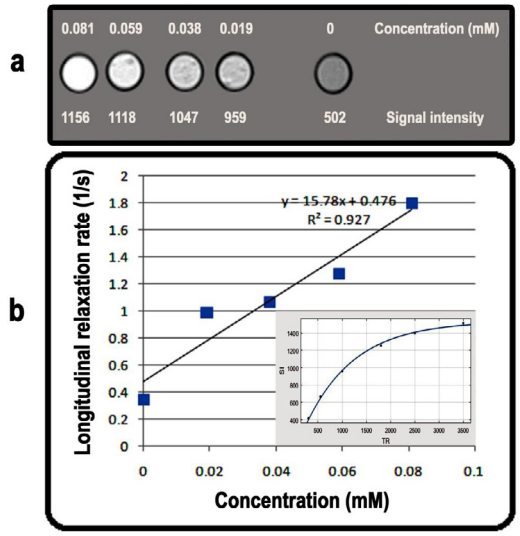

Fig 4. A) MR image at various Gd3+ concentrations of Gd/Go/alginate nanocomposite and B) Linear fitting plot of longitudinal relaxation rate versus Gd3+ concentration (inset: longitudinal relaxation time curve corresponding to signal intensity of test tubes demonstrated in Fig 4-A)

Fig 4-B demonstrates the longitudinal relaxation rate as a function of the gadolinium ion concentration. As can be observed, the relaxation rate was linearly correlated with the Gd^{3+} concentration; this linearity is essential to the use of each magnetic material in contrast-enhanced MRI. The slop of the line (i.e., relaxivity) shows the ability of the material to provide the MRI longitudinal contrast.

In the present study, the remarkably high longitudinal relaxivity value (15.78 $mM^{-1}.s^{-1}$) of Gd^{3+}/GO/alginate confirmed the provision of a strong positive contrast for the MR images using the nanocomposite. Furthermore, the high signal intensity values at the short repetition times could decrease the acquisition time of the image, thereby reducing the probability of motion artifact occurrence in further *in-vivo* MRI examinations. In the current research, another major implication of the high longitudinal relaxivity of the nanocomposite for further *in-vivo* imaging was the reduction of the administrated dose due to the significant increase in the proton relaxivity.

In the present study, the obtained longitudinal relaxivity using the Gd^{3+}/GO/alginate nanocomposite was significantly higher compared to the relaxivity values of the commonly used gadolinium chelates at the field strength of 1.5 T [23]. Table 1 shows the longitudinal relaxivity values of the nanostructures in the previous studies using gadolinium and graphene oxide complexes.

Table 1. Longitudinal relaxivity of nanostructures containing Gd and GO in previous studies at different field strengths

Contrast material	Longitudinal relaxivity ($mM^{-1}.s^{-1}$)	Field strength (T)	Reference
PFOB@PLA/GO/Gd-DTPA	4.66	9.4	24
GO/BaGdF5/PEG	4.8	0.5	25
Gd-NGO	7.59	7	26
GO–DTPA–Gd	10.8	11.7	13
GO-DOTA-Gd	14.2	11.7	14
Present study	15.78	1.5	-

According to the information in this table, the relaxivity of the nanocomposite was higher compared to the other findings in this regard. It is notable that longitudinal relaxivity depends on several parameters, such as the strength of the applied magnetic field and size and chemical structure of the nanoparticle/nanocomposite. Since these parameters may vary in the present study with the previous studies, the proper comparison of the reported longitudinal relaxivity in various studies may not be possible. As is known, longitudinal relaxivity typically decreases with increased field strength. However, accurate molecular design may still lead to very high relaxivity [3]. Additionally, the type and thickness of coating materials influence the value of longitudinal relaxivity. In some studies, gadolinium chelates have been used with variable effects on relaxivity [13, 14, 24]. It is also notable that in case of the agents with multifunctional theranostic applications [24, 25], there are other imaging or therapeutic materials that could affect relaxivity.

CONCLUSION

In this study, the Gd^{3+}/GO/alginate

nanocomposite was prepared and characterized in order to study the relevance between the longitudinal relaxation rate and gadolinium concentration. According to the results, the Gd^{3+}/GO/alginate nanocomposite had high cell viability at higher concentrations up to 75 µg/ml. Furthermore, the conjugation of Gd^{3+} ions to the GO nanosheet as a carrier resulted in shorter longitudinal relaxation times and more significant signal changes. A linear correlation was also observed between the longitudinal relaxation rate and gadolinium concentration with the high slope indicated that the Gd^{3+}/GO/alginate nanocomposite could potentially provide excellent brightness in MR images.

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