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Sensing the delivery and endocytosis of nanoparticles using magneto-photo-acoustic imaging

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ABSTRACT

Many biomedical applications necessitate a targeted intracellular delivery of the nanomaterial to specific cells. Therefore, a non-invasive and reliable imaging tool is required to detect both the delivery and cellular endocytosis of the nanoparticles. Herein, we demonstrate that magneto-photo-acoustic (MPA) imaging can be used to monitor the delivery and to identify endocytosis of magnetic and optically absorbing nanoparticles. The relationship between photoacoustic (PA) and magneto-motive ultrasound (MMUS) signals from the in vitro samples were analyzed to identify the delivery and endocytosis of nanoparticles. The results indicated that during the delivery of nanoparticles to the vicinity of the cells, both PA and MMUS signals are almost linearly proportional. However, accumulation of nanoparticles within the cells leads to nonlinear MMUS-PA relationship, due to non-linear MMUS signal amplification. Therefore, through longitudinal MPA imaging, it is possible to monitor the delivery of nanoparticles and identify the endocytosis of the nanoparticles by living cells.

1. Introduction

With a size of about 100 to 10000 times smaller than human cells, nanoparticles offer unprecedented potentials to interact with biomolecules and to revolutionize disease diagnosis and treatment. As an example, nanoparticles have recently attracted significant attention as the vehicle to deliver and release drugs and therapeutic agents to the disease site [9,28] to achieve highly localized therapeutic strategies. Such localized therapeutic applications ultimately necessitate a targeted intracellular delivery and availability of the nanoparticles to specific target cells [7,9,28]. The interaction between nanoparticles and target cells often includes two steps: delivery and accumulation. During the delivery step, the nanoparticles (administrated through the blood stream or directly injected to the site of interest) are delivered to the vicinity of target cells via different mechanism such as enhanced permeability and retention (EPR) effect [36]. Second, the delivered nanoparticles accumulate within the target cells through endocytosis, which is one of the primary membrane translocation mechanisms for nanoparticles entry into the living cells [15]. Fully understanding the interactions between nanoparticles and cells is an enormous advancement towards evaluating the utility of molecularly targeted nanoparticles in both diagnostic and therapeutic applications. In an ideal scenario, nanoparticles which are utilized as biosensors must be activated or generate signal only when they have entered the cells through endocytosis. However, in real applications, signals are generated not only from the endocytosed nanoparticles but also from the nanoparticles in the vicinity of the cells; and it is extremely difficult to differentiate between these signals. Most molecular imaging modalities have limited sensitivity and resolution to specifically sense the endocytosis of nanoparticles into living cells mostly due to similar signature of the obscuring signal generated by the delivered yet not endocytosed nanoparticles [4,43]. Therefore, imaging strategies capable of detecting nanoparticle delivery and sensing of their intracellular endocytosis is highly desirable.

Magneto-photo-acoustic (MPA) imaging was developed based on the integration of ultrasound (US) [6], photoacoustic (PA) [3,13,27,39,41], and magneto-motive ultrasound (MMUS) [18–22,26] imaging modalities [11,31–33,35]. By utilizing magneto-plasmonic nanoparticles as imaging contrast agent, MPA can provide imaging information at cellular and molecular level with a high resolution and sensitivity [10,33]. In our previous studies, we

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have reported a phenomenon that the intracellular accumulation of magnetic nanoparticles generates larger MMUS signals than the same concentration of isolated nanoparticles [22,23]. Furthermore, we demonstrated that MPA imaging could differentiate between the isolated nanoparticles in the vicinity of the cells and the accumulated nanoparticles within the cells [34]. In MPA imaging, the amplitude of the PA signal is proportional to the concentration of optical absorbers, i.e. magneto-plasmonic nanoparticles. Although accumulation of plasmonic metallic nanoparticles could increase the local heating effect and thus introduces linear and even nonlinear amplification of PA signal in aggregated and accumulated nanoparticles [25], such phenomenon may not happen for some types of nanoparticles such as dye-doped silica-coated magnetic particles [1,24]. On the other hand, when such nanoparticles are endocytosed, confined in lysosomes, and form intracellular aggregates with much larger size than isolated nanoparticles [5,8,12,38], the MMUS signal is amplified significantly. In other words, the MMUS signal from nanoparticles depends on both the concentration of nanoparticles and their functional state (i.e., isolated or endocytosed/aggregated) [22,23]. Therefore, the ratio between MMUS and PA (MMUS/PA) signals represents the magnetically induced motion from the unit concentration of nanoparticles. Such ratio, monitored over time, could identify both the delivery of nanoparticles and endocytosis of nanoparticles by cells.

Our previous work provided an initial framework for non-invasive detection of endocytosis of nanoparticles using MPA imaging [34]. In the current study, we further evaluate the utility of MPA imaging, coupled with an analytical method, to monitor nanoparticle delivery and to detect the endocytosis process. Herein, we propose a longitudinal analysis of MPA signal to monitor accumulation and endocytosis of nanoparticles within the cells. To achieve this goal, cell-tissue mimicking phantoms were designed to closely mimic the realistic in vivo scenario, in which some of the nanoparticles were endocytosed by the cells of interest, while the rest of the isolated nanoparticles were present in the vicinity of the cells. Two in vitro sets of experiments were designed to simulate the delivery and endocytosis of nanoparticles. The MPA imaging experiments were performed and the relationship between PA and MMUS signals were analyzed to identify the delivery and cellular accumulation processes.

2. Materials and Methods

2.1. Synthesis of magnetic nanoparticles as MPA contrast agents

Citrate-capped magnetite (Fe_3O_4) nanoparticles with optical absorption at the visible wavelength and large magnetic susceptibility (56 emu/gr Fe) were used as contrast agent in our MPA imaging studies. The citrate-capped Fe_3O_4 nanoparticles were synthesized through a phase transfer reaction between tri(ethylene glycol)-coated Fe_3O_4 nanoparticles in ethanol and an aqueous solution of 14 mg/mL sodium citrate (Sigma-Aldrich) in nano-pure water [33,37]. The volume ratio between the tri(ethylene glycol)-coated Fe_3O_4 solution and the sodium citrate was 1:1. The tri(ethylene glycol)-coated Fe_3O_4 nanoparticles were synthesized by the thermal decomposition of 1 g of iron (III) acetylacetonate ($\geq$99.9% trace metals basis, Sigma-Aldrich) in 20 mL tri(ethylene glycol) (Sigma-Aldrich) at ~250 °C for four hours (Maity et al., 2009). Prior to the phase transfer reaction, the obtained tri(ethylene glycol)-coated Fe_3O_4 nanoparticles were cleaned in 0.25 mL batches. A mixture of 0.25 mL Fe_3O_4 nanoparticles, 0.75 mL ethanol, and 1 mL ethyl acetate was centrifuged at 14,000 g for half an hour. A black NP pellet was obtained after decanting the supernatant. The cleaning step was repeated three times, and the obtained pellet of cleaned Fe_3O_4 nanoparticles was re-suspended in 0.25 mL ethanol. Then, the desired volumes of cleaned Fe_3O_4 nanoparticles in ethanol and the sodium citrate in water solution were mixed together and shaken at 500 rpm overnight, allowing the phase transfer reaction. In this reaction, the Fe_3O_4 nanoparticles' tri(ethylene glycol) surface layer was replaced with citrate ions. The citrate-capped Fe_3O_4 nanoparticles were obtained by centrifuging the reaction solution in Millipore 50 kDa Amicon Ultra-15 Centrifugal Filter Units at 3,000 g for 15 minutes. The obtained nanoparticles were re-suspended with nano-pure water and re-filtered four times. Finally the filtered citrate-capped Fe_3O_4 nanoparticles were re-suspended in 1 × PBS solution. The size of the synthesized Fe_3O_4 nanoparticles were measured as approximately 7.5 nm by using a transmission electron microscope (Fig. 1A). As shown previously, the Fe_3O_4 nanoparticles possessed strong optical absorption in visible wavelengths and high magnetic susceptibility [33]. In this study, the synthesized SPIO nanoparticles does not show plasmonic effect that can potentially interfere with hypothesis behind this study. These contrast agents do not have absorption peak within near infrared (NIR) region and absorb relatively high at 532 nm. Therefore, PA imaging experiments were performed at 532 nm wavelength.

2.2. Cell-nanoparticles tissue mimicking phantoms

The macrophages (J774 A1) cells were incubated with citrate-capped Fe_3O_4 nanoparticles for 24 hours. Then the harvested macrophages were washed three times with phosphate buffered saline (PBS). Cellular uptake, measured using inductively coupled plasma mass spectrometry (ICP-MS), was approximately 6.7×10^6 citrate-capped Fe_3O_4 nanoparticles (7.65 pg Fe_3O_4 nanoparticles) per cell. The TEM image of macrophages with endocytosed nanoparticles is shown in Fig. 1(B).

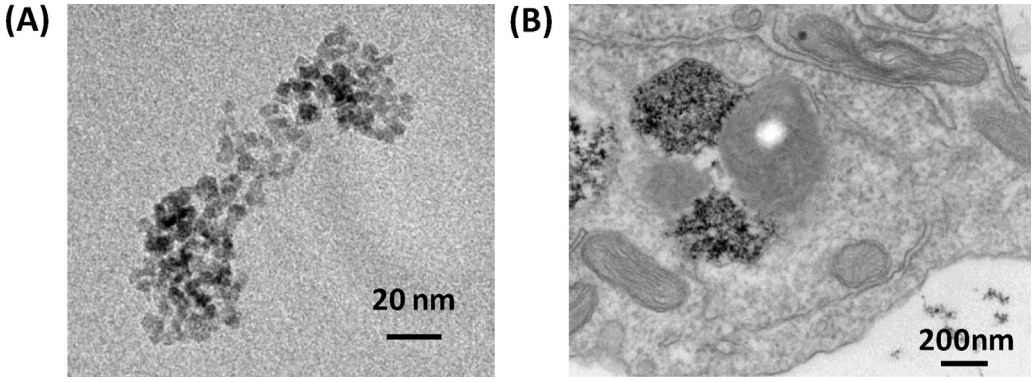

Fig. 1. Transmission electron microscopy (TEM) image of (A) Fe_3O_4 nanoparticles, (B) macrophages with endocytosed Fe_3O_4 nanoparticles.