

Preliminary In Vitro Study of Ultrasound Sonoporation Cell Labeling with Superparamagnetic Iron Oxide Particles for MRI Cell Tracking

Runyang Mo¹, Shuyu Lin¹, Gongzheng Wang¹, Yu Wang², Ed X. Wu³

Applied Acoustic Institute, Shaanxi Normal University, Xi'an, Shaanxi, 710062, China¹

College of Life Science, Shaanxi Normal University, Xi'an, Shaanxi, 710062, China²

Laboratory of Biomedical Imaging and Signal Processing, The University of Hong Kong, Hong Kong SAR, China³

Corresponding author: ewu@eee.hku.hk; mmrriycn@snnu.edu.cn

Abstract Vibration caused by ultrasonic waves can change the structure of cell membrane and enhance its permeation. In the last decade, a new ultrasound-aided method, sonoporation, has been proposed and utilized to transmit target molecules (such as drugs and DNA) into cells for therapy. The objective of this study was to investigate the method of loading nanometer-sized superparamagnetic iron oxide particles into Sarcoma 180 cells by sonoporation without chemical agents. The SPIO nanoparticles were prepared in our laboratory by means of classical coprecipitation and the formation of Fe₃O₄ crystal in SPIO nanoparticles was confirmed by x-ray diffraction analysis with its other characteristics assessed by magnetic hysteresis loops and size distribution. Cell labeling with SPIOs using sonoporation was successfully demonstrated in vitro for sarcoma180 cell suspensions from ICR mice. The labeling efficiency and viability were evaluated by Prussian blue staining. Such sonoporation technique can be employed for rapid labeling of various cells for MRI visualization of their spatiotemporal activities in vivo upon transplantation.

I. INTRODUCTION

Cell tracking using magnetic resonance imaging (MRI) is one of the methods to visualize carcinomatosis or pathology study in living animals. Since native cells are not detectable by MRI, it is necessary to label cells with contrast agent for cellular imaging. The most popular agents for MR cell tracking are superparamagnetic iron oxides (SPIOs) [1]. At present, the most widely used method of loading magnetic nanoparticles into cells is by coating the iron oxides with polycations, facilitating particles binding to anionic cell membranes, followed by internalization of the iron oxides complexes with electrostatic forces. However, cells must be incubated or cultured for extended

time periods (from a few hours to several days). Electroporation has been recently proposed and demonstrated for magnetic labeling [2]; Electroporation uses high voltage electrical pulses to make cell membranes transiently permeable permitting cellular uptake of foreign macro-molecules. Because its lack of target specificity and safety concerns, electroporation has limited applications in vivo.

Sonoporation, which uses ultrasonic waves instead of electrical pulses, can transiently enhance the permeability of cell membranes, facilitating the flux of foreign macromolecules into cells [3-6]. Furthermore, it has been demonstrated that ultrasound assisted by encapsulated microbubbles could make cell membranes temporarily "open", delivery drugs into cells through acoustic cavitation. The ultrasound technique associated with this process is often called sonoporation [7]. Furthermore, sonoporation, in contrast with electroporation, is relatively safe and can be focused to a specific location as desired.

In this study, we hypothesize that sonoporation can facilitate the intracellular loading of SPIOs. An experimental study for cell suspensions has been performed to observe changes in cells during sonoporation in presence of SPIOs. Labeling efficiency and viability were also evaluated.

II. METHODOLOGY

A. Preparation of SPIOs

Superparamagnetic iron oxides (SPIOs) colloid suspension was prepared with a slightly modified Massart method [8,9]. A mixture of 15 mL 0.8 M FeCl₃ solution and 10 mL 0.8 M FeCl₂ solution was added to a 500 mL three-neck flask containing 150 mL distilled water under stirring (200 rpm) and N₂ atmosphere at room temperature. Then, 55 mL 1 M

sodium hydroxide solution was added. After the addition, the stirring speed was elevated to 450 rpm, and the stirring lasted for another 30 min. The reaction system was then heated to 70 °C under vigorous stirring and further reacted for another 30 min. Finally, the system was cooled down to room temperature, and the iron oxide particles were separated magnetically. The magnetic particles collected were washed with water to receive a neutral SPIOs suspension. The solid content in the suspension is about 6680 $\mu\text{g}\cdot\text{ml}^{-1}$.

B. Ascites S180 Tumor Cells Preparation

ICR mice (18-22 g) were injected with 0.1 mL of tumor cells (S180 cell line, 1×10^7 cells $\cdot\text{mL}^{-1}$). The ascites cells were aspirated from the abdomen of such tumor-bearing mouse at 7-10 days post S180 inoculation and harvested by centrifugation (1500 rpm, 5min). The harvested cells were washed twice using normal saline, then, resuspended in normal saline at $1\text{-}5\times 10^6$ cells $\cdot\text{mL}^{-1}$ for further use. Before treatment, the cell viability was checked by trypan blue dye exclusion in a hemacytometer to ensure that cell viability was over 98%.

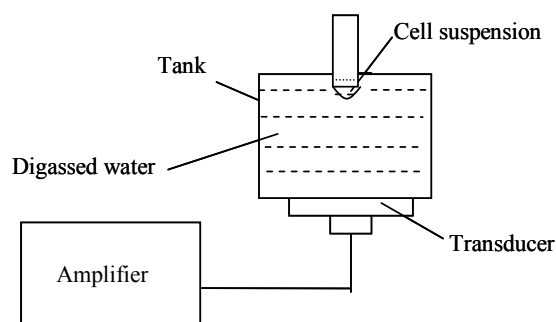


Fig.1. Sketch of the experimental setup. An ultrasound transducer in diameter of 47 mm and a focal length of 76 mm was submerged at the bottom of a plastic container filled with degassed water. The frequency of the ultrasound was 1.43 MHz.

C. Ultrasound Exposure and Sonoporation Labeling

Cells which suspended in phosphate-buffered saline (PBS) at a density of $1\text{-}5\times 10^6$ cells $\cdot\text{mL}^{-1}$ were placed into a sterile 1 mL polystyrene round-bottom tube and randomly divided into six groups. The sample tubes were fitted into a water bath (4 °C). SPIOs were added at a range of

22.3-66.80 $\mu\text{g Fe/mL}$.

Optimization studies of ultrasound exposure were first performed to determine the various physical parameters, such as acoustic transmitted frequency, ultrasound exposure duration, and ultrasound intensity. Note that cell membrane poration induced by ultrasound exposure has been studied in gene transfer and drug delivery [6,7]. In those studies, the probe which transmitted frequency in 1-2 MHz with intensity less than $3\text{ W}\cdot\text{cm}^{-2}$ were widely used.

In this study, cells were sonoporated using a system shown in Fig. 1 [10]. The ultrasound transducer (with diameter 47 mm) with a resonant frequency of 1.43 MHz in a continuous wave mode was used to convert the electrical power measured by the AG 1020 Ultrasound amplifier (T&C Power conversion, Inc., Rochester, New York, USA) into acoustic power. The ultrasound intensity were adjusted by the liquid crystal display console of amplifier and the output electrical power from the amplifier was $2.0\text{ W}\cdot\text{cm}^{-2}$.

Experiments were performed to determine the effects of (i) different ultrasound exposure duration (10, 30, 60s); (ii) various SPIO concentrations (22.27, 44.53, 66.80 $\mu\text{g}\cdot\text{mL}^{-1}$); (iii) cell incubation with SPIOs suspension under no ultrasound exposure. After insonification, all samples were incubated at 37 °C for 10 min. Then the samples were fixed with 2.5% (v/v) glutaraldehyde at 4 °C for 15 min.

D. Evaluation of Labeling Efficiency

SPIO uptake by S180 cells following sonoporation was evaluated by Prussian blue staining. Presence of SPIOs in cytoplasm was confirmed by dense blue staining.

E. Cellular Viability

After ultrasound exposed, cells were incubated at 37 °C for 10 min, Trypan blue staining was employed to assess the viability of the cells labeled with SPIOs.

III. EXPERIMENT RESULTS

A. The Physical and Magnetic Properties of SPIOs Nanoparticle Prepared in Our Laboratory

The main characteristics of SPIO nanoparticles were assessed by x-ray diffractogram (Fig.2); magnetic hysteresis loops (Fig.3) and size distribution (Fig.4).

The formation of Fe_3O_4 crystal in SPIO nanoparticles was confirmed by x-ray diffraction analysis (Fig.1). 20 mL extraction of SPIOs suspension were air dried, and analyzed with X-ray diffraction technique (Model Rigaku D/MAX-III, Japan). The X-ray diffraction spectra were taken for 2θ angles from 10° to 80° at a scan rate of $0.02^\circ/\text{min}$

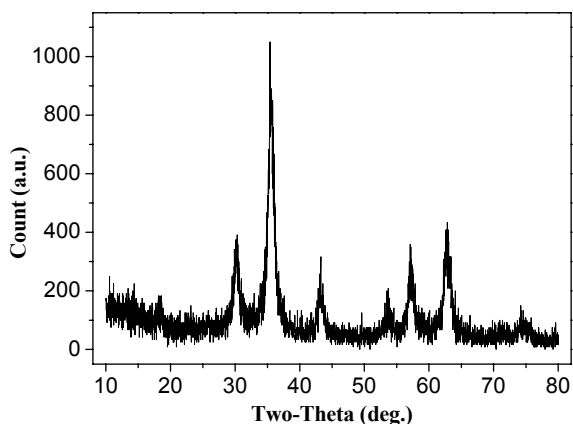


Fig. 2. X-ray diffractogram of SPIO nanoparticles

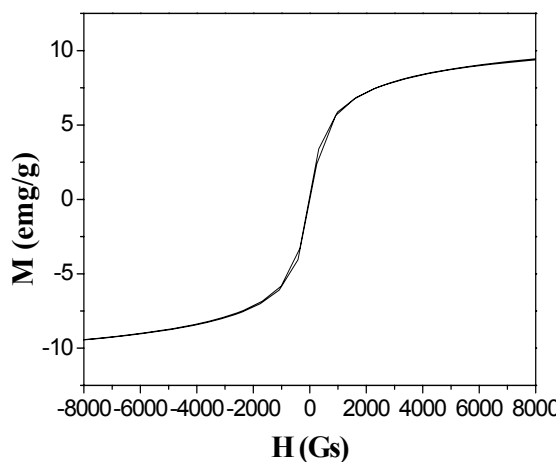


Fig. 3. Magnetic hysteresis loops of SPIO nanoparticles

Fig.3 depicts the magnetic hysteresis loops of the superparamagnetic Fe_3O_4 nanoparticles, revealing that the magnetic hysteresis loops of the superparamagnetic Fe_3O_4 nanoparticles is almost a coherent curve. This indicates that the SPIO nanoparticles are soft magnetic materials, with high magnetic conductance rate, low residual magnetism, low coercive force, and low reluctivity [11]. Equivalently, SPIOs are superparamagnetic materials. In other words, the SPIO nanoparticles can response sensitively to a quickly changing external magnetic field.

Fig.4 shows the size distribution of the iron core. The average diameter of magnetic particles was 63.9 nm.

B. Cell Labeling Effect

SPIOs concentration in cell suspension was $22.27 \mu\text{g}\cdot\text{mL}^{-1}$, During 10 min of cell incubation at 37°C , 1.43 MHz continuous wave with power intensity of $2.0\text{W}\cdot\text{cm}^{-2}$ was applied for 30 s. The S180 cells stained with Prussian blue in Fig. 5 demonstrate the uptake of SPIO particles as a

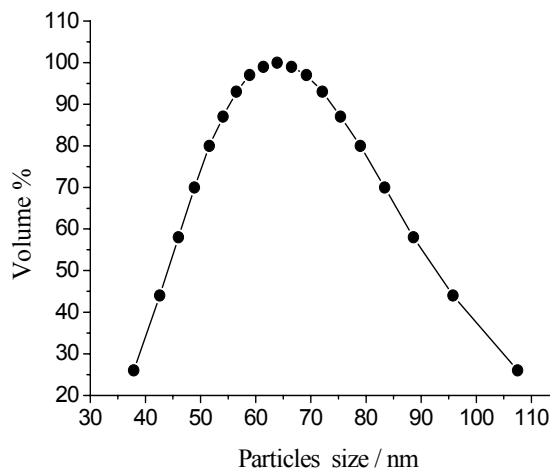


Fig. 4. Size distribution of SPIO nanoparticles

result of sonoporation treatment. Note that SPIO particles are visible as blue iron deposits (Fig.5a). We also found that no cell could be labeled if incubating time was less than 30 min and cells were not treated with sonoporation.

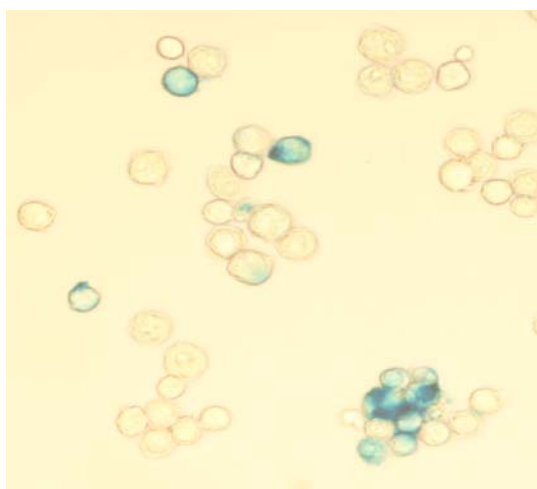
C. Cellular Viability for Different Concentrations of SPIO and Durations of Ultrasound Exposure

With 30 s ultrasound exposure and 10 min incubation with SPIOs of $66.80 \mu\text{g}\cdot\text{mL}^{-1}$, cellular iron uptake increased with little change in cell viability. However, $44.53 \mu\text{g}\cdot\text{mL}^{-1}$ or $22.27 \mu\text{g}\cdot\text{mL}^{-1}$ SPIO concentrations did not lead to apparent increase of the amount of cellular iron uptake.

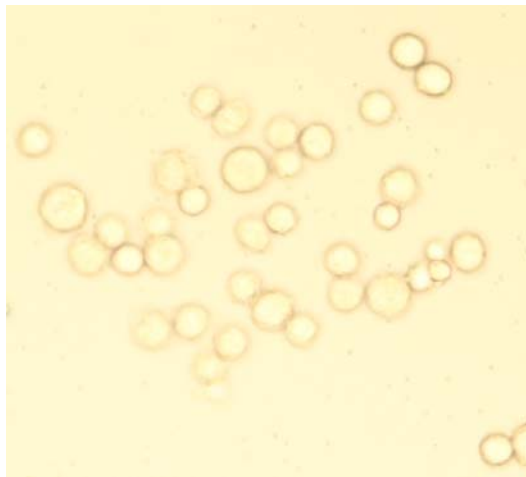
IV. DISCUSSION

The preliminary results of this study demonstrate that sonoporation is a powerful tool, potentially capable of labeling cells with nanoparticles for in vivo MRI. Sonoporation procedure is rapid (with the total procedure lasting for about 15 min), and can be spatially selective if necessary via focused ultrasound. The method should be

widely applicable as sonoporation instrument is ubiquitous in most laboratories. Sonoporation is a physical method, in which an ultrasound field is applied. This allows for direct and instant loading of SPIO nanoparticles into cells. However, several issues remain to be addressed in future studies, such as optimal SPIO concentration and ultrasound exposure conditions, as well as direct observation of the pores induced in cell membrane during sonoporation.



(a)



(b)

Fig.5. Fe uptake by ultrasound-treated S180 cells and untreated (original magnification: 40×). (a) Blue staining of ultrasound-treated S180 cells with Prussian blue, showing SPIO loading. (b) Untreated S180 cells, showing no SPIO loading.

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