PLGA-PEG base magnetic nanocapsule for contrast-enhanced MR imaging and focused ultrasound-triggered drug delivery

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Abstract

Focused ultrasound-triggered drug delivery by using the ultrasound and magnetic nanocapsules is a promising strategy to target and release drugs toward tumour sites under magnetic resonance imaging (MRI) in cancer therapy. In this work, we co-encapsulated superparamagnetic iron oxide (SPIO) and chemotherapeutic drug into the nanocapsules which is made of Poly(Lactide-co-Glycolide)(PLGA)-Poly(Ethylene Glycol)(PEG) polymer to form the magnetic multifunctional nanocapsules as an efficient MRI contrast agent as well as an anticancer drug carrier. The nanocapsules exhibited narrow size distribution and smooth spherical morphology with a diameter of 204 ± 9.8 nm. The *in vitro* results demonstrated that the nanocapsules could significantly enhance the MR imaging because of the magnetic nanoparticles and exhibit excellent biocompatibility. Moreover, an irreversible drug release under focused ultrasound triggering was achieved and the tumour cell killing efficiency of nanocapsules was significantly higher than the control groups. The proposed nanocapsules could be a useful alternative for cancer diagnosis and treatment by the focused ultrasound-triggered drug delivery. Moreover, we believe that if the magnetization of the nanocapsules is enhanced, it will be utilized as a nanorobot for tumour therapy which can be actively controlled by an external magnetic field.

1 Introduction

The development of stimulus responsive nanoscale systems for anti-cancer drug delivery which allows the simultaneous execution of therapeutic and diagnostic approaches have received great attention, as these systems can differentially increase accumulation at target lesions and remarkably decrease systemic toxicity [1]. A variety of external physical stimuli, including near-infrared (NIR) light, electric field, magnetic field, and ultrasound have been applied to trigger and enhance the localized cancer therapy [2-5]. Among them, focused ultrasound has become a promising one because of its non-invasiveness facile regulation of tissue penetration depth, and the absence of ionizing radiations.

Recent advances in nanotechnology and biomedicine permits the design of multifunctional nanoplatform that combines the function of stimuli-responsive, diagnostic and therapeutic with a single nanostructure, using various inorganic or organic materials. Organic Poly(Lactide-co-Glycolide) (PLGA) is a biocompatible and biodegradable copolymer and has been approved by the US FDA for use as scaffold in tissue engineering and in various drug and gene delivery [6]. However, pure PLGA nanoparticles potentially unstable and tend to form large aggregates, probably due to the hydrophobic surface. The circulation time and passive targeting to the tumour could be improved by modifying the nanoparticle surface with hydrophilic, electrically neutral polymers such as Poly(Ethylene Glycol) (PEG), as a result of the enhanced permeability and retention (EPR) effect [7]. In addition, superparamagnetic iron oxide (SPIO) has been used as negative contrast agent for T2*-weighted magnetic resonance imaging because of their high relaxivity and excellent contrast enhancement [8].

In this study, we developed PLGA-PEG based magnetic nanocapsules as an effective nanoplatform for focused ultrasound triggered anti-cancer drug delivery and MR imaging. The synthesis of PLGA-PEG was confirmed by nuclear magnetic resonance (^{1}H NMR) analysis and the physical and chemical properties of the magnetic nanocapsules were characterized. Moreover, the capability for MR imaging contrast was evaluated. Finally, we investigated the focused ultrasound triggered drug releasing and cytotoxicity of magnetic nanocapsules.

2 Materials and methods

2.1 Synthesis of superparamagnetic iron oxide (SPIO)

Hydrophobic SPIO were synthesized using a chemical co-precipitation method [9]. Briefly, 10 mmol iron(III) chloride and 5 mmol iron(II) chloride were dissolved in 24 ml of an hydrochloride aqueous solution (HCl 1M). Then, the solution was added dropwise to an aqueous solution of NaOH 1M containing 3g of oleic acid with vigorous mechanical stirring for 60 min under the protection of dry nitrogen at 80℃. The black precipitate was mag-

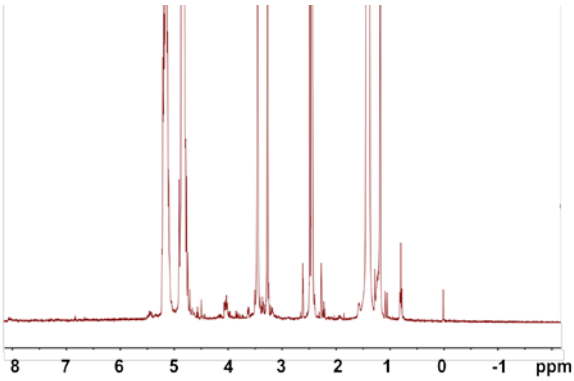

Figure 1 ^{1}H NMR spectrum of PLGA-PEG polymer

netically separated, washed three times using absolute ethanol and then dispersed in 20 ml of methylene chloride The solution was then placed in an ultrasonic bath for 10 min and centrifuged (2000 rpm, 10 min) to remove the undispersed residue.

2.2 Synthesis of PLGA-PEG copolymer

PLGA-PEG block copolymer was synthesized by the reaction between carboxylic group of PLGA and amine group of PEG using the zero-length crosslinker dicyclohexyl carbodiimide (DCC) in the presence of N-hydroxysuccinimide (NHS) [10]. Briefly, PLGA was activated by DCC and NHS in methylene chloride for 24 h before being conjugated with PEG-amine for 12 h. The resultant solution was purified by filtering and precipitated by dripping into ice-cold diethyl ether. The precipitated product was dissolved in dimethyl sulfoxide (DMSO) and dialyzed against DI water for 2 days (MWCO: 10,000) to obtain PLGA-PEG with high purity. Then, the final product was freeze dried for 2 days.

2.3 Preparation of magnetic nanocapsules

Magnetic nanocapsules were fabricated by a double water/oil/water (W/O/W) emulsion solvent evaporation method [11]. Briefly, 50 mg of SPIO were mixed with the organic solution of the PLGA-PEG polymer solution (100 mg PLGA-PEG in 2 mL methylene chloride) by stirring. Then, 0.3 mL of doxorubicin (DOX) solution (10 mg DOX dissolved in 300uL deionized water) was added to the organic phase, and the mixture was emulsified by sonication for 1 min. Subsequently, 10 mL of poly (vinyl alcohol) (PVA) solution (5 wt%) was added to this initial emulsion, and the mixture was then further sonicated in ice bath for 5 min. The resultant double emulsion was diluted in 50 mL of PVA solution (0.5 wt%) under mechanical stirring at room temperature for overnight to evaporate the organic solvent. Finally, the centrifugation and washing with deionized water process was repeated three times.

2.4 Physical and chemical characterization

The PLGA-PEG synthesis was confirmed by nuclear magnetic resonance (^{1}H NMR, 400 MHz, AVANCE III HD 400; Bruker, Billerica, MA) analysis. The mean diameter and size distribution of magnetic nanocapsules were assessed using a size and zeta potential analyser (ELS-8000; Otsuka Electronics, Osaka, Japan). The amount of SPIO in the polymer shell was quantified by using inductively coupled plasma atomic emission spectroscopy (ICP-OES) (PE-3300DV; Perkin Elmer, Norwalk, Connecticut), and the morphology and structures of these magnetic nanocapsules were studied using a scanning electron microscope (SEM, SS-550; Shimadzu, Kyoto, Japan) and a transmission electron microscopy (TEM, FE-TEM, JEM-2100F, JEOL Ltd, Tokyo, Japan) The magnetization property was further evaluated using a vibrating sample magnetometer (VSM, Lake Shore Cryotronics 7404, Westerville, OH) at room temperature (25 ℃) The DOX loading efficiency was analysed using high performance liquid chromatography (HPLC, Agilent LC1100, Agilent, Tokyo, Japan) system.

2.5 *In vitro* MRI imaging experiment

Magnetic nanocapsules were diluted to final concentrations of 0, 0.025, 0.05, 0.1, 0.2, 0.4, 1.2 mM in PBS (pH 7.4) and imaged on a MRI Scanner (Philips Achieva 3.0T TX, Philips Medical Systems, Netherlands). T2*-weighted (T2*WI) images were obtained using the following parameters; repetition time (TR) = 72 ms, and echo time (TE) = 9 ms, Flip angle = 45° , slice thickness of 3.0 mm. The MRI signal intensity within the region of interest (ROI) was measured

2.6 *In vitro* DOX release study

In order to assess the DOX release profiles under focused ultrasound triggering, *in vitro* drug release experiment was performed. The focused ultrasound system setup and experimental parameters for focused ultrasound triggering drug release were similar to those in our previous studies [12]. 10 mg of magnetic nanocapsules were distributed in PBS (5 mL, pH 7.4) and placed in a dialysis bags (MWCO; 3,500 Da), which were transferred in a reservoir of 50 mL of PBS with stirring at 100 rpm at 37℃. The magnetic nanocapsules were then triggered using a 925 kHz transducer with acoustic pressure of 800 kPa, pulse length of 1000 cycle, and the pulse reputation frequency (PRF) of 20 Hz. At appropriate intervals, 1 ml of dialysate was removed from the sample for later analysis and 1 ml of fresh PBS solution was then added back to the reservoir to keep a constant volume. The concentration of DOX in each sample was determined using HPLC analysis. Control experiments were performed using the same conditions, but without focused ultrasound triggering.

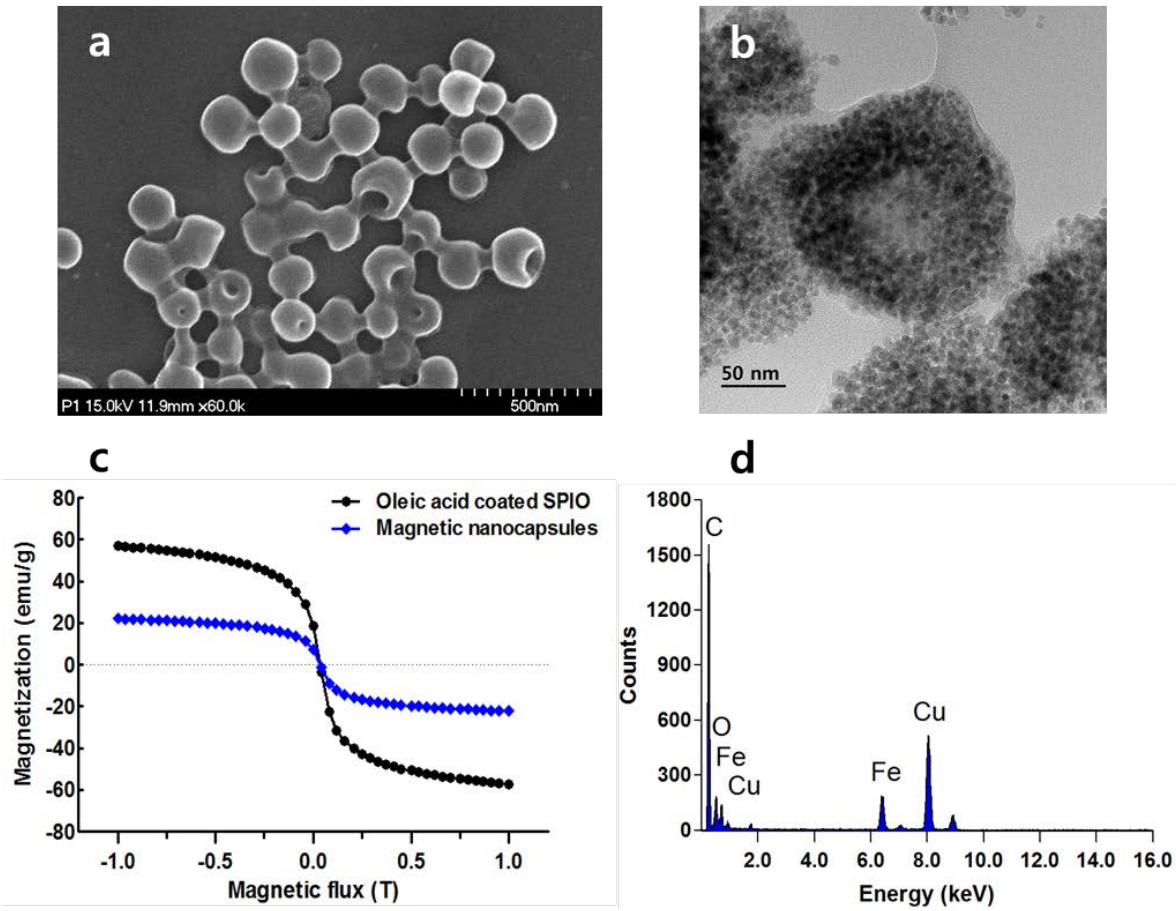

Figure 2 a) SEM image of magnetic nanocapsules. b) TEM image of magnetic nanocapsules. c) VSM curve of magnetic nanocapsules. d) EDS spectrum of Fe elements in the magnetic nanocapsules.

2.7 Cytotoxicity tests

The cytotoxicity of free DOX, DOX loaded magnetic nanocapsules and focused ultrasound triggered magnetic nanocapsules was determined by a MTT cell proliferation assay. Briefly, 4T1 breast cancer cells were seeded at a density of 3×10^4 cell per well in 48-well plates. Next day, the used medium was replaced with different formulation solutions: fresh medium, pure magnetic nanocapsules and free DOX, and DOX loaded magnetic nanocapsules with DOX concentration of 10 μg/ml. After 12 h, the cell wells were placed on sample holder at 37℃ and the certain cell line was triggered with focused ultrasound for 3 min. After further incubation for 12 h, supernatants were removed. Then, the wells were washed twice with PBS and incubated with DMEM containing MTT (5mg/ml) for an additional 4 h. The MTT solution was removed and dimethyl sulfoxide (DMSO) was added to dissolve the cells for spectrum measurement. The absorbance at 570 nm was measured by a microplate reader (Thermo Scientific, Waltham CA).

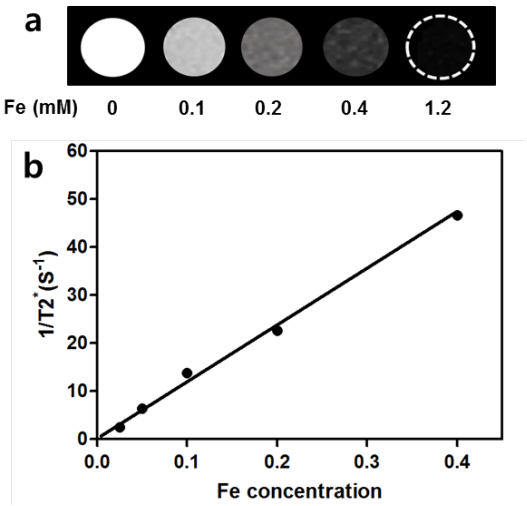

Figure 3 a) and b) show the T2-weoghted MR images and the transverse relaxation rate (1/T2*) of magnetic nanocapsules with different Fe concentrations.

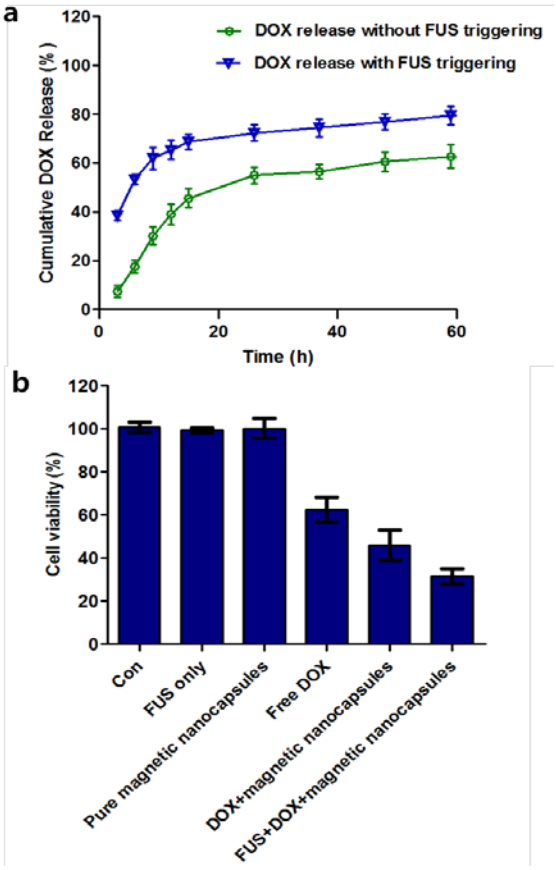

Figure 4 a) *In vitro* DOX release profiles with or without focused ultrasound triggering. b) Cell cytotoxicity of magnetic nanocapsules with or without focused ultrasound triggering against 4T1 cells.

3 Results and discussion

3.1 Characterization of PLGA-PEG polymer

The synthesis of PLGA-PEG was confirmed by ^{1}H-NMR analysis. Chemical shifts were expressed to part per million (ppm) relative to the standard NMR solvent (DMSO) signal. Fig. 1 demonstrates the proton peaks associated with PLGA-PEG polymer. The peaks at 1.6 ppm and 5.2 ppm are corresponding to –CH3 and –CH- protons of PLA block. The peak at 4.8 ppm belongs to the –CH2- protons of PGA block. The peak at 3.6 ppm is attributed to –CH2- protons of PEG block. The results confirmed the successful synthesis of PLGA-PEG copolymer [13].

3.2 Characterization of magnetic nanocapsules

In this study, magnetic nanocapsules with iron oxide embedded in polymer shell (PLGA-PEG) were formulated by water-oil-water emulsion solvent evaporation process. The amount of SPIO encapsulated in the magnetic nanocapsules determined by ICP-OES method was 2.8 mg/ml, and the payload of drug measured with HPLC system was 242 µg/mL. The SEM image of magnetic nanocapsules (Fig. 2a) shows that the magnetic nanocapsules exhibited a smooth and uniform spherical morphology. The presence of hydrophobic SPIO in the polymer shells of magnetic nanocapsules was determined by the enhanced contrast manifested as dark domains in TEM image (Fig. 2b). The polymer shell was also visible as grey areas surrounding black spots {SPIO}. Fig. 2c shows the magnetization properties of these magnetic nanocapsules which are measured by VSM. The saturation magnetization of magnetic nanocapsules was about 22 emu/g. The energy dispersive X-ray spectrum (EDS) for magnetic nanocapsules clearly indicated the presence of Fe elements in the polymer shell further demonstrating the success of incorporation of SPIO nanoparticles (Fig.2d).

3.3 *In vitro* MRI experiments

To assess the ability of magnetic nanocapsules for enhancing magnetic resonance imaging, the relaxation rate ($1/T2^{*}$) was measured by a clinical MRI instrument. Fig. 3 shows the $T2^{*}$ weighted MR images of magnetic nanocapsules with various iron concentrations. The MR signal intensity decreased as iron concentration increased demonstrating that magnetic nanocapsules have produced the magnetic resonance contrast on transverse photon relaxation time-weighted sequence. The *in vitro* results indicated that magnetic nanocapsules could serve as the potential MRI contrast agents.

3.4 Focused ultrasound triggered DOX release profiles

The DOX release from the magnetic nanocapsules could be triggered by focused ultrasound sonication, due to the cavitation, mechanical pressure, and acoustic streaming effect induced by focused ultrasound [12,14]. The release profiles of DOX from magnetic nanocapsules with or without focused ultrasound triggering were measured to evaluate the effects of ultrasound triggering on DOX releasing. As shown in Fig. 4a, each releasing profile was demonstrated by the percentage of released DOX as a function of time. The result shows that the DOX release from magnetic nanocapsules without triggering was less than 63% at 60 h. However, with focused ultrasound triggering, the releasing rate was significantly faster than those of controls; approximately 79% of the DOX was released after 60 h. In addition, the time for 50% of the DOX to be released from magnetic nanocapsules with or without triggering was about 6 h and 24 h, respectively. This result implies that the DOX release rate can be controllably triggered with focused ultrasound, and the DOX can be specifically deposited at the tumour sites under the triggering

3.5 Cytotoxicity study

Finally, the tumour cell killing effect of magnetic nanocapsules combined with focused ultrasound was studied (Fig. 4b). The cell survival rates of control group and magnetic nanocapsules only group showed no significant differences. However, the drug loaded magnetic nanocapsules group showed obvious tumour cell killing effect. In addition, the drug loaded magnetic nanocapsules with focused ultrasound triggering group showed much higher tumour killing effect. This result was probably caused by the triggered drug release and enhanced cellular drug uptake due to the focused ultrasound sonication [15].

4 Conclusion

In this study, we have successfully developed a doxorubicin and superparamagnetic nanoparticles co-loaded PLGA-PEG based magnetic nanocapsules for both magnetic resonance imaging and focused ultrasound triggered anti-cancer drug delivery. The capability of magnetic nanocapsules for MRI contrast agent was evaluated and a high transverse relaxivity (1/T2*) of 112 $mM^{-1}s^{-1}$ was achieved. Furthermore, irreversible triggered drug release and significantly enhanced tumour cell killing effect was obtained. Therefore, we believe that the magnetic nanocapsules could be a promising nanoplatform for MR imaging and focused ultrasound triggered anti-cancer drug delivery. Furthermore, the magnetic nanocapsules exhibits relatively high magnetic properties than that reported by other groups [6, 7], therefore, it will be applied in the nanorobot systems for cancer therapy which can be actively controlled by an external magnetic field.

5 Acknowledgments

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6 Reference

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7月6日（星期四）Thursday，July 6, 2017， Room: M3-04

主题/Topic		类人/仿生/足式机器人，微型机器人 Biologically Inspired Robotics, Micro / Nano Robotics
会议室/Room 1		M3-04
Time	Number	Paper Title
09:40-10:00	No.87	用于基于细胞的治疗剂固定的新型永磁体阵列的模拟 Simulation of Novel Permanent Magnet Array for Cell-based Therapeutic Agent Fixation
		Kyungmin Lee
		School of Mechanical Engineering, Chonnam National University, Korea
10:00-10:20	No.84	多功能胶囊内窥镜新型电磁驱动系统：可行性研究 Novel Electromagnetic Actuation System for Multifunctional Capsule Endoscopes: A Feasibility Study
		Manh Cuong Hoang
		School of Mechanical Engineering, Chonnam National University, Gwangju, Korea Medical Microrobot Center, Robot Research Initiative, Chonnam National University, Gwangju, Korea
10:20-10:40	No.85	PLGA-PEG 基磁纳米胶囊，用于对比增强 MR 成像和聚焦超声触发药物递送 PLGA-PEG base magnetic nanocapsule for contrast-enhanced MR imaging and focused ultrasound-triggered drug delivery
		Zhen Jin
		School of Mechanical Engineering, Chonnam National University, Gwangju, Korea
10:40-11:20	Keynote Speech	生物医学微/纳米机器人 Biomedical Micro/Nano Robotics
		Prof. Jong-Oh Park
		IFR Executive Board Member Director, Medical Microrobot Center Director of Robot Research Initiative Professor of Chonnam National University, Korea
11:20-12:00	Keynote Speech	人工智能驱动智能机器人工业走向创新经济 AI Driven Intelligent Robotics Industry towards Innovation Economy
		罗仁权教授 Prof. Ren C. Luo
		Chair Professor & life distinguished professor at National Taiwan University Director of International Center of Excellence on Intelligent Robotics and Automation Research in National Taiwan University Member of EU Industrial Advisory Board, Taiwan Editor-in-Chief, IEEE Transactions on Industrial Informatics (Impact Factor 4.708)
12:00-12:50		午餐 Lunch Break

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