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Superparamagnetic PLGA-iron oxide microcapsules for dual-modality US/MR imaging and high intensity focused US breast cancer ablation

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ABSTRACT

Organic/inorganic, hybrid, multifunctional, material-based platforms combine the merits of diverse functionalities of inorganic nanoparticles and the excellent biocompatibility of organic systems. In this work, superparamagnetic poly(lactic-co-glycolic acid) (PLGA) microcapsules (Fe_3O_4/PLGA) have been developed, as a proof-of-concept, for the application in ultrasound/magnetic resonance dual-modality biological imaging and enhancing the therapeutic efficiency of high intensity focused ultrasound (HIFU) breast cancer surgery *in vitro* and *in vivo*. Hydrophobic Fe_3O_4 nanoparticles were successfully integrated into PLGA microcapsules by a typical double emulsion evaporation process. In this process, highly dispersed superparamagnetic Fe_3O_4/PLGA composite microcapsules with well-defined spherical morphology were obtained with an average diameter of 885.6 nm. The potential of these microcapsules as dual contrast agents for ultrasonography and magnetic resonance imaging were demonstrated *in vitro* and, also, preliminarily *in vivo*. Meanwhile, the prepared superparamagnetic composite microcapsules were administrated into rabbits bearing breast cancer model for the evaluation of the *in vivo* HIFU synergistic ablation efficiency caused by the introduction of such microcapsules. Our results showed that the employment of the composite microcapsules could efficiently enhance ultrasound imaging of cancer, and greatly enhance the HIFU ablation of breast cancer in rabbits. In addition, pathological examination was systematically performed to detect the structural changes of the target tissue caused by HIFU ablation. This finding demonstrated that successful introduction of these superparamagnetic microcapsules into HIFU cancer surgery provided an alternative strategy for the highly efficient imaging-guided non-invasive HIFU synergistic therapy of cancer.

1. Introduction

Organic poly(lactic-co-glycolic acid) (PLGA) nano-/micro-particles have been extensively employed in the applications of drug delivery, tissue engineering and molecular imaging [1–3]. Comparatively, the inorganic superparamagnetic Fe_3O_4 nanoparticles have found their application in either T_1- or T_2-weighted magnetic resonance imaging (MRI), magnetically targeted drug delivery and hyperthermia [4–9]. By combining the advantages of PLGA microcapsules and magnetic Fe_3O_4 nanoparticles, organic/inorganic hybrid composite biomaterials with broader and more feasible applications could be produced compared to standalone applications of either PLGA or magnetic Fe_3O_4 nanoparticles.

High intensity focused ultrasound (HIFU) ablation has been shown as a successful non-invasive, complication-free treatment, and is also known as focused ultrasound ablation, or focused ultrasound surgery (FUS) [10]. HIFU was introduced by Lynn for the first time in the 1940s in the performance of neurologic surgery [11]. Over the past few decades, HIFU as a promising non-invasive modality in the treatment of solid tumors has been developed rapidly [12–15]. Abundant scientific investigations have concluded that HIFU ablation was safe, effective and feasible and could be used for clinical destruction of tumor tissues [16–18].

Among all cancers, breast cancer is one of the most common type of cancer and the second leading cause of cancer-related death among women [19]. Surgery has been the standard of

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care, offering the chance of complete cure in selected breast cancer patients by tumor removal. However, breast is an important superficial organ to women, and breast conservation is indeed a wise and an attractive option in patients with breast cancer. Among the numerous existing techniques, HIFU is a non-invasive technique. HIFU supplies a focused ultrasound beam of high energy in a manner that analogous to the way a magnifying glass can be used to focus light, to damage targeted tissue without affecting surrounding tissue. However, the therapeutic efficiency of HIFU is relatively low to some diseases, especially those with a larger volume or deeper lesion, as ultrasound energy attenuates exponentially with the increase of depth in tissues, and energy also can be reduced in the areas that are adjacent to high-speed blood flow [20,21]. To obtain the desired therapeutic goal, high ultrasound power must be employed. Such high acoustic power can cause damage to the normal tissue in the ultrasound propagation channel, causing severe side effects, such as transient pain, skin burns, and nerve injury [22–24]. Obtaining high therapeutic efficiency with low therapeutic power in HIFU therapy remains as an unmet challenge. Previously, we have successfully introduced nano-biotechnology into the non-invasive therapeutic field by employing mesoporous silica-based multifunctional nanocapsules for the synergistic MRI-guided HIFU liver cancer surgery. The results have demonstrated that the introduction of imaging and synergistic agents could efficiently locate the cancer tissue for further ultrasound focusing, and the PFH encapsulated mesoporous nanocapsules can significantly enhance the HIFU therapeutic efficiency [25,26]. However, the biocompatibility of inorganic silica nanoparticles remains to be further explored and demonstrated.

Herein, we would like to introduce an organic/inorganic hybrid platform based on Fe_3O_4-containing PLGA microcapsules (Fe_3O_4/PLGA) to enhance the HIFU therapeutic efficacy and simultaneous MR/US dual-modality biological imaging for HIFU guidance. PLGA is a preferential candidate as carrier material, because of its high stability, biodegradability and biocompatibility, which ensures acoustic properties and prolonged circulation time, and safety for *in vivo* applications and clinical translation [27,28]. In addition, superparamagnetic Fe_3O_4 nanoparticles have been transported to clinical imaging applications. The reason for the integration of MRI and ultrasonagraphy was based on two major considerations. First, both ultrasonography and MRI are widely used diagnostic modalities for various experimental and clinical applications. Second, ultrasonography is the commonly used real-time imaging tool for the guidance of HIFU therapy, while it has limitations such as bone will affect its imaging. MRI is well known for its high quality imaging and it will not be affected by bone, while it is not real-time. The integration of MRI and ultrasonagraphy should be able to give more imaging information for precise diagnosis and imaging guidance.

2. Materials and method

2.1. Preparation of the superparamagnetic microcapsules

PLGA microcapsules encapsulating Fe_3O_4 (Fe_3O_4/PLGA) were fabricated by a double emulsion (water/oil/water) evaporation process. First, 200 μL solution (1% w/v) of nano-sized Fe_3O_4 particles (10 mg/mL, size 10 nm, Ocean) was added to 2 mL CH_3Cl dissolving 100 mg PLGA (50:50, MW = 20,000, Daigang, China). The mixture was emulsified using an ultrasonic probe (SONICS & MATERALS Inc; USA) at 130 W for 30 s after adding 200 μL deionized water. Then, the above emulsified solution was poured into 10 mL poly(vinyl alcohol) (PVA, MW = 25,000, Sigma) solution (5% w/v) and homogenized (FJ300-SH, Shanghai, China) within 5 min for the second emulsion. The final emulsion was mixed mechanically for 2 h to extract CH_3Cl. Subsequently, the solution was centrifuged at 3500 rpm for 3 min, the supernatant was discarded, and the precipitate was washed by deionized water. The process of centrifugation was repeated three times. Finally, the precipitate was collected and stored at 4 °C for further use.

2.2. Structural characterization of Fe_3O_4/PLGA microcapsules

The size distribution and the morphological characterization were estimated by a light microscope (Olympus CKX41; CANADA) and a scanning electron microscope (SEM , Hitachi, S-3400N; Japan). A Laser Particle Size Analyzer System (Zeta SIZER 3000HS; Malvern, USA) was used to acquire the mean size of Fe_3O_4/PLGA microcapsules. The magnetic properties were investigated by a Physical Property Measurement System (PPMS, Model 6000, Quantum Design) at a temperature of 300 K.

2.3. Animal models

All animal experiments were approved by our animal ethics committee. Tumor-bearing rabbit with a VX2 tumor in the thigh was obtained from the laboratory of Ultrasound Engineering Institute of Chongqing Medical University. Other New Zealand white rabbits as recipient animals, weighing 2.0–2.5 kg, between 2 and 3 months-old were purchased from and bred in the Animal Center of Chongqing Medical University under standard conditions according to the Institute's environmental guidelines. All experiments and procedures were performed under complete anesthesia.

2.3.1. Implantation of VX2 liver tumors

Under sterile conditions, the tumor tissue with fish-meat-like appearance was excised from the tumor-bearing rabbit, washed with normal saline, and then subdivided into small tissue pieces about 1 mm^3. Rabbits as recipient animals were anesthetized by intramuscular injection of 3% pentobarbital solution (1 mL/kg), and the abdomens of the rabbits were depilated with 8% Na_2S solution, then fixed in prone position and routinely disinfected. A median incision was made below the xiphoid process to expose the middle lobe of liver, where a hole about 5 mm deep was made using ophthalmological forceps, then one tissue piece was implanted into the hole. After bleeding was stopped, the abdominal wall was sutured. Skin incision was disinfected before, 500,000 units streptomycin was intramuscular injected for three consecutive days to prevent infection.

2.3.2. Implantation of VX2 breast tumors

The breast tumor model was developed according a reported method [29]. Briefly, tumor tissue was excised from tumor-bearing rabbit with similar procedures to section 2.3.1. The tumor tissue was soaked in 20 mL Hanks' balanced salt solution. Then, the tumor was sheared to small masses with a size of approximately 0.5–1.0 mm in diameter. The final suspension was extracted into a 20 mL syringe, and 1 mL tumor tissue suspension was injected into the mammary gland of rabbits underneath the left second nipple. As well, 500,000 units streptomycin was used after the tumor implantation.

2.4. In vitro ultrasound imaging of Fe_3O_4/PLGA microcapsules

A preliminary evaluation of the ultrasound contrast behavior of Fe_3O_4/PLGA microcapsules was carried out using a gel mold. Fe_3O_4/PLGA microcapsules at concentrations of 100, 50, 25, 12.5 mg/mL in 1 × PBS were imaged at various mechanical indexes (MI, from 0.7 to 0.1). Images of 1 × PBS and air were acquired serving as background controls. The samples were scanned using an ultrasonic diagnostic instrument (12 L; IU22; Philips Medical Systems, Bothell, WA) in conventional B mode.

2.5. In vitro and in vivo MRI assessment of Fe_3O_4/PLGA microcapsules

2.5.1. In vitro MRI

Fe_3O_4/PLGA microcapsules were diluted to final concentrations of 0, 2.5, 5, 10, 25, 50 μg Fe/mL in 1 × PBS and imaged on a Philips Achieva 3.0T TX MR Scanner (Philips Medical Systems, Netherlands). T_2*-weighted images were acquired using the following parameters: fast field echo (FFE), TR = 72 ms, and TE = 9 ms, Flip = 45°, FOV = 180 mm, slice thickness = 3.0 mm. The MRI signal intensity (SI) within the region of interest (ROI) was measured.

2.5.2. In vivo MRI

21 days after tumor inoculation in the liver, rabbits ($n = 24$) with detectable liver cancer were used to carry out the MRI experiment. T_2-weighted images were acquired before, at 30 s and 5 min after intravenous injections (1 mL/kg) of Fe_3O_4/PLGA microcapsules (3.1 mg Fe/mL), saline and pure (without Fe_3O_4) PLGA microcapsules. To acquire T_2-weighted images, turbo spin echo (TSE) sequences were run at TR values of 2500 ms, TE = 80 ms, Flip = 90°, FOV = 160 mm, slice thickness = 4.0 mm. The SI was determined by comparing SIpre, and SIpost (30 s, 5 min) of the ROI in the liver parenchyma.

2.6. HIFU ablation

In this experiment, JC200 HIFU tumor ablation equipment (Chongqing Haifu Technology, Chongqing, China) was used. This equipment consists a therapeutic US unit and a diagnostic US unit under the control of a central processing system. The