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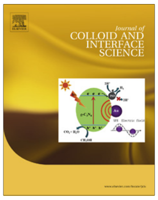

Regular Article

Structural regulation of self-assembled iron oxide/polymer microbubbles towards performance-tunable magnetic resonance/ultrasonic dual imaging agents

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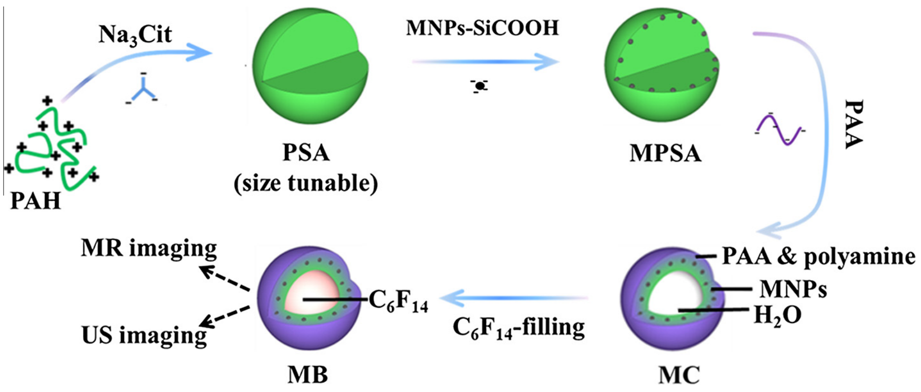
GRAPHICAL ABSTRACT



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ABSTRACT

Fe_3O_4/polymer hybrid microcapsules were prepared *via* a template-free route which is based on polyamine-salt aggregates (PSAs) self-assembly approach. The measurements of transmission electron microscopy (TEM) indicated that the diameter and shell thickness of the microcapsules could be tuned by varying the experimental conditions, such as the concentration of reactants and evolution time employed during the PSA assembly. The results of vibrating sample magnetometer (VSM) demonstrated that the magnetic nanoparticles content of the synthesized microcapsules was tunable and all samples exhibited superparamagnetic behavior. After filling appropriate perfluorocarbon into the inner cavities of the microcapsules, the biomedical applications of the resultant microbubbles, including ultrasonic imaging (USI) and magnetic resonance imaging (MRI), were studied *in vitro*. It showed that the synthesized magnetic microbubbles possessed both strong ultrasound contrast enhancement capability and high relaxation rate. The excellent acoustic and magnetic properties of these self-assembled microbubbles ensure that the Fe_3O_4/polymer hybrid microbubbles have great potential as MRI/USI dual-modality contrast agents.

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1. Introduction

As diagnostic medical techniques, magnetic resonance imaging (MRI) and ultrasonic imaging (USI) have been successfully employed in various clinical applications [1–3]. However, both of these technologies have their advantages and drawbacks at the same time, such as sufficient spatial resolution but somewhat low sensitivity in the case of MRI and high sensitivity but insufficient spatial resolution in the case of USI [1,4–6]. Therefore, MRI/USI dual contrast agents are of great significance in combining and improving the complementarity of these two imaging techniques. In most of the previous studies, MRI and ultrasonic contrast agents have exhibited their own apparently different features in composition and structure, respectively [7–9]. In particular, hydrophilic superparamagnetic iron oxide nanoparticles (Fe_3O_4) have advantages in acting as T_2-weight MRI contrast agents [10,11], while polymer microcapsules with perfluorocarbon-filled cavities (microbubbles) play an important role as USI agents [12,13]. Recently, the use of Fe_3O_4/polymer hybrid microbubbles as MRI/USI dual contrast agents has gained increased attention and expanded the possibilities of MRI/USI dual-modality imaging [14–16].

To fabricate the microcapsules, double emulsion approach and layer-by-layer (LBL) assembly are typical strategies among the various established methodologies [17–23]. Unfortunately, double emulsion approach suffers from a key limitation in that hydrophobic polymers are the only choice available for this strategy [15,18,19]. As a template-based self-assembly method, LBL technique provides a distinct advantage over other strategies by allowing the precise control of the sizes of the microcapsules. Nevertheless, this synthetic procedure is somewhat time-consuming and material-wasting, during which the deposition of oppositely charged polyelectrolyte pairs is tediously repeated, ending with the removal of template [20,22,23]. Recently, Wong et al. [24–26] reported a template-free self-assembly strategy known as the polyamine-salt aggregate (PSA) assembly to obtain inorganic-particle/polymer hybrid microcapsules, through which they successfully synthesized silica/polymer hybrid microcapsules. Inspired by this discovery, we exploited and built on the PSA assembly to generate hybrid microcapsules bearing entrapped Fe_3O_4 magnetic nanoparticles (MNPs). Furthermore, by filling the microcapsules with poorly-diffused perfluorocarbon, microbubbles were prepared and exhibited great potential as MRI/USI dual imaging agents [27]. However, further studies should be performed in order to elucidate the mechanism of this template-free method, to evaluate the universality of the PSA assembly strategy and the tunability of the microcapsule sizes.

As we know, the diameter and thickness of microbubbles as determined by the size of their microcapsular precursors exert significant impact on their performance as ultrasonic imaging agents [16,28]. In the process of ultrasonic imaging, sound waves are transmitted into and received by an object. This process is known as echogenicity, and is proportional to the frequency of sound and the density of the reflecting material relative to that of its surroundings [29]. The nature of the reflecting material in the cavity of microbubbles is the crucial factor that directly influences the intensity of ultrasonic imaging [3,30]. In addition, the enhancement of T_2-weighted MRI is vitally linked with the content of superparamagnetic Fe_3O_4 nanoparticles [31–34], which is inextricably related to the diameter and thickness of the microbubbles. Moreover, the *in vivo* circulation and clearance of these particles correspond strongly with their diameters [35–37]. For particles as large as 4–5 μm, the clearance generally occurs in the liver, spleen and lungs, while particles smaller than 20 nm are rapidly excreted by the glomeruli of kidneys. Besides, when the particle size comes to the range from 20 nm to 1 μm, the clearance mechanisms are less efficient and the circulation time is extremely prolonged. In summary, the diameter and thickness of microbubbles are two critical parameters that significantly affect the biomedical imaging and metabolism.

In this contribution, we fabricated microcapsules or microbubbles on the basis of PSA assembly and incorporated novel ligand-modified Fe_3O_4 nanoparticles into these particles in order to demonstrate the universality of the aforementioned template-free self-assembly technique. The results indicated that the microcapsules were successfully generated through PSA self-assembly and microbubbles with perfluorocarbon-filled cavity exhibited great potential as USI/MRI dual-modality contrast agents. Moreover, the diameter and shell thickness of Fe_3O_4/polymer hybrid microbubbles could be tuned by varying the concentration of the reactants and aging time during the process of PSA assembly, yielding different effects on their performance as MRI/USI agents.

2. Materials and methods

2.1. Materials

Anhydrous ferric chloride, anhydrous ethanol, sodium oleate, *n*-hexane, acetone, trisodium citrate dehydrate, fluorescein sodium salt and glacial acetic acid (AR) were purchased from Sinopharm Chemical Reagent Co., Ltd. Oleic acid (OA, 90%), 1-octadecene (90%), a poly(allylamine) solution (PAH, $M_w = 65000$ Da, 20 wt% in water), poly(acrylic acid) (PAA, $M_w = 1800$), tetradecafluorohexane (C_6F_{14}), *N*-(trimethoxysilylpropyl) ethylene diamine triacetic acid sodium salt (SiCOOH, 45 wt% in water), *N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC, 99%, AT) and *S*-acetylthioglycolic acid *N*-hydroxysuccinimide ester (NHS, 95%, TLC) were purchased from Sigma-Aldrich and used as received.

2.2. Preparation of the self-assembled Fe_3O_4/polymer hybrid microbubbles

2.2.1. Preparation of the SiCOOH-capped Fe_3O_4 magnetic nanoparticles (MNPs-SiCOOH)

The preparation of oleic acid-capped Fe_3O_4 magnetic nanoparticles (MNPs-OA) was performed according to the approach reported by Park et al. [38]. In a typical procedure, the iron-oleate complex was synthesized by the reaction of iron chloride ($FeCl_3{\cdot}6H_2O$, 40 mmol) with sodium oleate (120 mmol) for 4 h at 70 °C. Subsequently, the reaction mixture containing the iron-oleate complex, oleic acid and 1-octadecene was heated to 320 °C at a constant heating rate of 3.3 $°C\ min^{-1}$. Then, this temperature was maintained to largely produce MNPs-OA for 30 min. After cooled to room temperature, the synthesized hydrophobic Fe_3O_4 MNPs were washed with acetone and *n*-hexane and subsequently precipitated *via* centrifugation. The washing and centrifugation was repeated five times.

In order to prepare hydrophilic SiCOOH-capped magnetic nanoparticles (MNPs-SiCOOH), the oleic acid moieties on the MNPs-OA surface were replaced with SiCOOH groups using a previously reported ligand exchange method [39,40], with some improvements incorporated to enhance the yield of the ligand exchange process. Generally, hydrophobic MNPs-OA (500 mg) was dispersed into hexane with the addition of 50 μL acetic acid. The solution was sonicated for 10 min prior to the addition of SiCOOH (6 mL). The mixture was continuously stirred for 48 h at 60 °C *via* magnetic stirring. After the surface modification