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Controlled magnetosomes: Embedding of magnetic nanoparticles into membranes of monodisperse lipid vesicles

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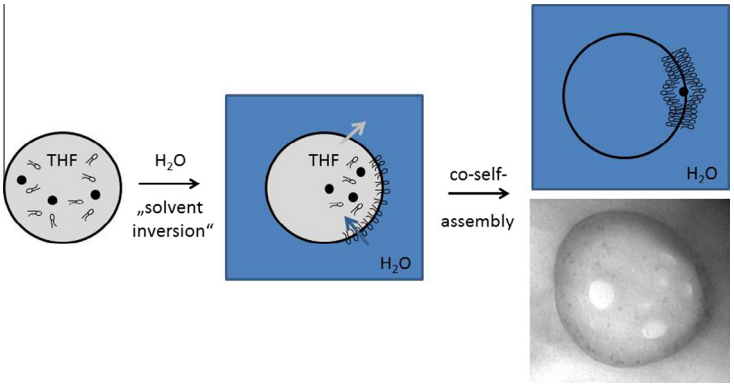
GRAPHICAL ABSTRACT



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ABSTRACT

Magnetic nanoparticle-containing capsules have been proposed for many uses, including triggered drug delivery and imaging. Combining superparamagnetic iron oxide nanoparticles (SPIONs) with existing liposome drug delivery technology is an enticing near-future prospect, but it requires efficient methods of synthesis and formulation compatible with pharmaceutical applications. We report a facile way of producing large, unilamellar, and homogeneously sized magnetoliposomes with high content of monodisperse, hydrophobic SPIONs integrated in the lipid membrane by use of a solvent inversion technique. For low lipid concentrations, unilamellar and monodisperse vesicles were obtained that became increasingly multilamellar with higher lipid fraction. Both, the co-self-assembled structure and loading content were significantly influenced by the purity of the nanoparticle shell. SPIONs with homogeneous shells of nitrodopamine-anchored hydrophobic dispersants could be quantitatively loaded up to 20%w/w, while SPIONs also containing residual physisorbed oleic acid exhibited a loading cut-off around 10%w/w SPIONs accompanied by drastic changes in size distribution. Lipid acyl chain length crucially influenced the formation and resultant stability of the loaded assemblies. The formation of nanoparticle-loaded vesicles is exemplified in different biologically important media, yielding ready-to-use magnetoliposome formulations.

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1. Introduction

Large unilamellar liposomes (~100–200 nm in diameter) comprise some of the most successful delivery systems in clinical use and are heavily researched for development of new drug delivery systems [1–4]. The advantages of liposomes are manifold [1,5,6]. Foremost, they possess a natural excellent biocompatibility and enable transport of hydrophilic cargo in their large aqueous lumen as well as hydrophobic and amphiphilic drugs in the lipid bilayer [1,7]. The composition and functionality of the lipid membrane can be easily tuned, including addition of charged lipids for transfection [8], PEG-lipids to create so-called stealth liposomes [9,10] with strongly reduced clearance rates in vivo, and tags that provide specific interactions with particular tissues or cells [3,6,10]. An important consideration when applying lipid vesicles for drug delivery is that efficient encapsulation and circulation can lead to inefficient or slow release. Rapid release at the site of action is desired to reach a concentration within the therapeutic range. Destabilizing the lipid membrane to increase its permeability, however, leads to premature drug release during circulation and short shelf-life. The self-assembled nature of lipid membranes offers many possibilities to make the release profile dependent on changes in the environment, thereby utilizing them for stable encapsulation and circulation, but letting a local change in the physical environment increase the release rate at the target [3]. Thermoresponsive liposomes are a well-researched example already in clinical use; they exploit small changes in tissue temperature between the blood stream and cancer tissue to increase the release rate by enhancing membrane permeability through a lipid composition exhibiting the melting phase transition slightly higher but close to the tissue temperature. The small temperature difference and the broad transition range strongly limit this approach. Therefore, externally applied triggers such as light, ultrasound or magnetic fields have captured the imagination to devise new delivery strategies [7,11–15]. The to date perhaps most successful such strategy is to use nanoparticles interacting with an external field to induce a thermal phase transition that increases the permeability of the vesicle membrane [7,14].

Liposomes structurally including biocompatible superparamagnetic iron oxide nanoparticles (SPIONs) are an attractive alternative for such strategies [7,11,14,16]. SPIONs, in contrast to most other magnetic nanoparticles, offer the advantage of being hydrolytically degraded into constituent nontoxic ions [17–19] and are highly compatible with in vivo applications due to the low susceptibility of tissue to magnetic fields [7,17,18]. Additionally, they offer the possibility to simultaneously image and control biodistribution via magnetic field gradients remotely [20], which makes them attractive also as multi-purpose tools for guided drug delivery and bioimaging [21–23]. For all these applications, controlling the size distribution, aggregation state and number of encapsulated magnetic nanoparticles is a key issue since magnetostatic interactions govern the force in magnetophoresis and dictate spin–spin relaxation times which control magnetic resonance imaging contrast and heat dissipation [24,25]. Therefore, high quality nanoparticles [26], ultrapure and stable surface modifications [27] and control over the embedding efficiency and distribution during the assembly process are prerequisites for applications.

To date various preparation methods have been described for producing magnetoliposomes [4,5,28]. Co-incorporation of water soluble SPIONs and pharmaceutical agents in the liposome lumen was first demonstrated [11,29,30]. Major drawbacks have been shown for this approach. First, SPIONs that are not properly stabilized interact with the liposome membrane and cause leakage, but properly stabilized SPIONs take up large volume. Second, heating by SPIONs in the lumen to induce a thermal transition requires heating of the entire environment to change the permeability of the membrane due to the high thermal conductivity of water [11]. In contrast, hydrophobic SPIONs embedded in the lipid bilayer were shown to directly act on the capsule wall without requiring heating of the surrounding environment for release [11,16]. The drawback however is that the embedding efficiency heavily depends on particle size [31–33]. Small SPIONs can be contained in the membrane but SPIONs >5 nm are thought to lead to micelle formation. However, small SPIONs interact less with magnetic fields due to their lower magnetic moment ($\sim d^3$). It was also demonstrated that the stability and density of the hydrophobic coating of the nanoparticles decisively influences the permeability of the membrane and the stability of the liposomes [33]; excess physisorbed ligands can lead to severe leakage of encapsulated compounds [11,16]. Optimal magnetic liposome preparations therefore aim for high loading of monodisperse SPIONs, as large as can fit in the membrane, to maximize the efficiency of actuation; this requires a dense and stable hydrophobic coating. While the feasibility of such magnetoliposomes for release has been demonstrated [11,16], to date, control over high loading of monodisperse hydrophobic SPIONs in the membrane of liposomes has not been achieved. Previous methods used rehydration and extrusion to produce unilamellar vesicles, which is not compatible with current techniques for liposome drug formulation where carrier liquids with costly drug molecules and lipids are injected to ensure high encapsulation; a similar technique for magnetoliposome assembly compatible with high encapsulation efficiency in a large lumen is therefore desired.

We report such an easy and scalable way using solvent inversion to produce homogeneously sized, large and unilamellar magnetoliposomes with high and tunable content of monodisperse SPIONs embedded in the membrane. The magnetosomes are characterized with respect to their morphology and loading content in various media.

2. Experimental

2.1. Materials

Phospholipids were obtained from Avanti Polar Lipids, USA and used as received.

All chemicals (analytical grade) were purchased from Sigma–Aldrich and used without further purification.

Monodisperse 3.5 nm N-palmityl-6-nitrodopamide (P-NDA) capped superparamagnetic iron oxide nanoparticles (SPIONs) were synthesized as reported previously [27]. In brief, 200 mg as-synthesized SPIONs were purified by repeated pre-extraction in hot MeOH containing 1 mM oleic acid as stabilizer before exchanged in a mixture of 150 mg P-NDA in DMF:$CHCl_3$:MeOH = 6:3:1 for 3 h under nitrogen gas. Newly capped SPIONs were evaporated to the DMF fraction, precipitated by adding excess MeOH and collected via magnetic decantation. The particles were purified by threefold extraction in hot MeOH. Mixed dispersant SPIONs were post-coated with 100 mg P-NDA in minimal 2,6-lutidine for 48 h at 50 °C under inert atmosphere, evaporated to dryness and purified by hot MeOH extractions. SPIONs were lyophilized from THF:H_2O (1:5).

2.2. Vesicle preparation by solvent inversion

The respective amount of high-vacuum dried lipid (usually 5 mg) or respective nanoparticle-lipid mixes were dissolved in 1 ml anhydrous THF and dropwise (approx. 1 drop per second) added into 10 ml aqueous phase (ultrapure water or buffers) under constant magnetic stirring (400 rpm). THF was evaporated for 24 h