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Magnetically targetable microcapsules display subtle changes in permeability and drug release in response to a biologically compatible low frequency alternating magnetic field

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ABSTRACT

High frequency alternating magnetic fields (AMF) have been widely used as a non-invasive method to induce local hyperthermia for antitumor treatment and to efficiently trigger drug release from various carriers. However, few studies have exploited the potential of targeted drug delivery to healthy cells or tissue and the use of low frequency AMF (LF-AMF) for intracellular triggered release. To achieve this goal, doxycycline was delivered with the layer-by-layer (LbL) assembled magnetic microcapsules, and AMF with low frequency (50 Hz) was applied. The low frequency AMF had little effect on morphology of microcapsules, which upon exposure for 360 min caused no significant damage and had the advantage of minimizing heating effects. Nonetheless, microcapsule permeability increased as a function of exposure time when assessed using FITC-dextran (70 kDa) with the number of permeable microcapsules increased from 13.5% (20 min) to 52.8% (360 min). Increased permeability also enhanced *in vitro* doxycycline release in genetically engineered myoblast cells where EGFP expression is regulated by the tetracycline system, while targeted EGFP expression was observed by magnetically navigating the microcapsules to a site of interest. Upon LF-AMF exposure of 30 min, no cytotoxicity was observed, but intracellular doxycycline release was promoted and enhanced EGFP expression as demonstrated by EGFP fluorescence intensity measurement. This study reveals the possibility of targeted drug delivery and using LF-AMF as a non-cytotoxic intracellular trigger of drug release from microcapsules without alteration in cell viability.

1. Introduction

Efficient delivery of therapeutic agents to a specific target site is of great interest in disease treatment since the capability of concentrating a drug locally can reduce the overall dose and adverse effects of off-targeted molecules on normal cells or tissue [1,2]. The requirement of targeted delivery has motivated the design of various carriers in recent decades with the idea of navigating drug loaded carriers in a programmed route. To achieve this goal, tremendous efforts have been put into engineering the surface of carriers with antibodies, peptides, nucleic acids or other ligands to enhance their affinity to target sites [3,4]. These chemically based specific interactions have proven effective *in vitro*, however, non-specific uptake and clearance *in vivo* limit their performance [5]. Another effective approach is to utilize physical navigation with an external magnetic field. Carriers, such as micelles, liposomes, emulsions and microcapsules, can be functionalized with magnetic nanoparticles and directed to the target tissue with a magnetic field [6–8]. Among which, LbL microcapsules have advantages for targeted delivery. Their multilayered architecture can be constituted of bio-functional macromolecules, and magnetic nanoparticles can be incorporated into their shells. Magnetically targeted delivery of bioactive molecules including DNA with microcapsules has already been reported [9,10].

Once localized at the targeted site, release of substances from microcapsules can be triggered by an external alternating magnetic field (AMF) [11–13]. Many magnetic particles embedded in the microcapsule walls are sensitive to AMFs, including magnetite, strontium ferrite, manganese ferrite and others [14]. The vibration of magnetic

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particles corresponds to the frequency of the AMF and leads to relaxing of polyelectrolytes and loosening of capsule walls, which increases the permeability for encapsulated cargos. AMFs have been used for triggered release from other carriers, such as liposomes [15,16], micelles [17,18], polymer particles [19,20] and mesoporous silica spheres [21–23]. However, it is worth highlighting that in most of these cases the heating effect of magnetic particles under high frequency AMF causes carrier breakdown and molecule release [12]. Magnetically mediated hyperthermia has been employed to enhance cancer cell apoptosis, upon antitumor chemotherapy agent delivery [24]. Although the local heating at surface of magnetic particles decays with increasing distance from the surface [25], the lesser heating effect produced by low frequency AMF is preferred for the delivery of bioactive substances (*e.g.* enzymes, DNA) to normal tissues [26–28]. There is also study trying to minimize the heating effect of high frequency AMF by using liposomes with high melting point and reducing the AMF exposure level [29,30]. Moreover, it is known that the low frequency AMF (up to 150 Hz) can more efficiently increase the permeability of a polymer matrix [14].

In our studies we use doxycycline (Dox) a derivative of the antibiotic tetracycline as a model drug because we can monitor its activity in a sensitive bioassay based on gene expression regulation by the *tet-on* system [31]. Specifically, we use a tetracycline responsive promoter (P_{tet}) in combination with a responsive repressor *tet*R-KRAB and an optimised activator rtTA2SM2 [32]. Other studies have established light and pH regulated gene expression by chemically modifying doxycycline with a photoactivatable cage [33,34] or conjugating it to dendritic polymers *via* a pH-sensitive bond [35]. In our previous study, doxycycline was encapsulated into microcapsules, and a local sustained activity was observed by a fluorescent signal generated *via* the *tet-on* modulated EGFP expression system [36]. Magnetic microcapsules can be navigated under flow conditions [37], and high frequency AMF triggered release of cargo has been reported [12], but no intracellular release in response to external triggers has been reported. Therefore, we aimed to further control doxycycline delivery with magnetic microcapsules by exploiting the possibility of intracellularly triggering its release with low frequency AMF without affecting cell viability (Scheme 1).

2. Material and methods

2.1. Materials

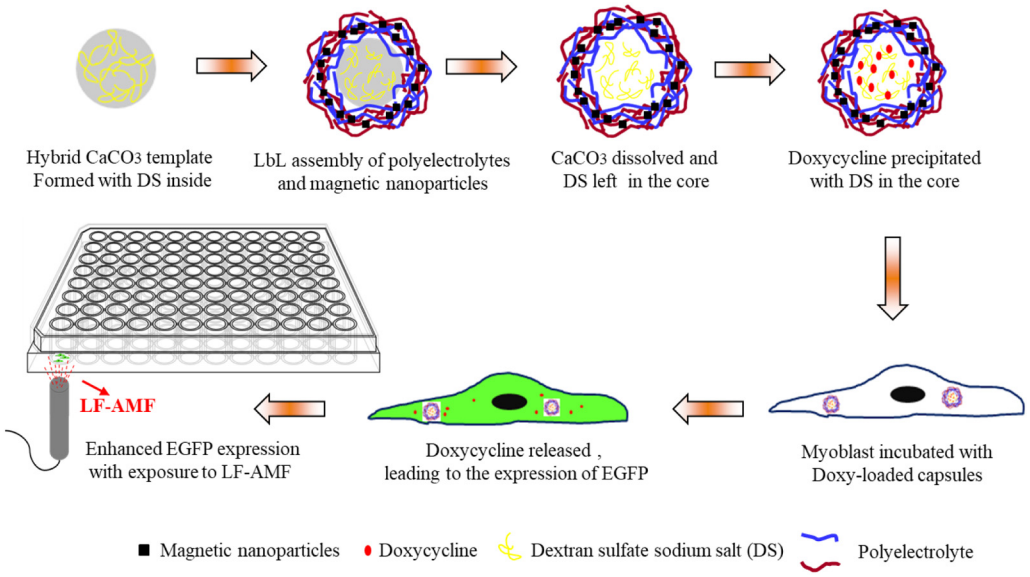
Poly(allylamine hydrochloride) (PAH, 56 kDa), poly(sodium 4-styrenesulfonate) (PSS, 70 kDa), dextran sulphate sodium salt (DS, 100 kDa), doxycycline hydrochloride, rhodamine B isothiocyanate, thiazolyl blue tetrazolium bromide (MTT), cell lysis reagent (CelLytic™ M), protease inhibitor cocktail, ethylenediaminetetraacetic acid disodium salt (EDTA), calcium chloride, sodium carbonate and all other salts were purchased from Sigma-Aldrich UK. Other biological reagents, including DMEM, PBS, foetal calf serum (FCS), penicillin, streptomycin and trypsin were purchased from Lonza Biologics Inc. (Newington, NH, United States).

2.2. Fe_3O_4 nanoparticles synthesis

Fe_3O_4 nanoparticles were synthesized by mixing 2.35 g $FeCl_3$ and 0.86 g $FeCl_2$ in 40 ml H_2O in a three-neck flask, which was placed in an oil bath and heated up to 80 °C in an argon atmosphere. The mixture was next stirred, while 5 ml NH_4OH was added slowly with a syringe. The reaction was maintained at 80 °C for 30 min and then 2 ml of 0.5 g/ml citric acid was added. The temperature was next raised to 95 °C and held for 90 min. The magnetic nanoparticles were dialysed against H_2O in a 14 kDa cut-off membrane for one week. The Fe_3O_4 nanoparticles were then characterized with TEM.

2.3. Microcapsule assembly and doxycycline encapsulation

Microcapsules were prepared by a well-known LbL self-assembly method with slight modification [36]. Briefly, hybrid $CaCO_3$ templates were prepared by pre-adding DS (20 mg/ml) to 0.33 M $CaCl_2$ solution and then precipitated with 0.33 M Na_2CO_3. After a triple wash with deionized H_2O, PAH (2 mg/ml) and PSS (2 mg/ml) were subsequently assembled on their surface. After assembly of five layers, the Fe_3O_4 nanoparticles were used as a negative sixth layer by the same procedure. For fluorescent visualization, the second outmost PAH layer was replaced with TRITC-PAH. Eight layers were deposited in total and $CaCO_3$ cores were then dissolved with 0.2 M EDTA solution, resulting in the microcapsule architecture - DS/$(PAH/PSS)_2$/PAH/FeNP/PAH-TRITC/PSS. In some instances, an additional PEI layer was also deposited as the outmost layer. As a control, microcapsules without



Scheme 1. Design of magnetic microcapsules for doxycycline delivery and LF-AMF triggered release.

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