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Effective delivery of immunosuppressive drug molecules by silica coated iron oxide nanoparticles

Jangsun Hwang[a], Eunwon Lee[b], Jieun Kim[b], Youngmin Seo[a], Kwan Hong Lee[a,b], Jong Wook Hong[b], Assaf A. Gilad[c,d], Hansoo Park[a], Jonghoon Choi[a,*]

[a] School of Integrative Engineering, Chung-Ang University, Seoul 156-756, Republic of Korea
[b] Department of Bionano Engineering, Hanyang University ERICA, Ansan 426-791, Republic of Korea
[c] Russell H. Morgan Department of Radiology and Radiological Health, Johns Hopkins University School of Medicine, Baltimore MD, USA
[d] Cellular Imaging Section, Institute for Cell Engineering, Johns Hopkins University School of Medicine, Baltimore, MD, USA

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ABSTRACT

Iron oxide nanoparticles have been used in a wide range of biomedical applications, including drug delivery, molecular imaging, and cellular imaging. Various surface modifications have been applied to the particles to stabilize their surface and to give them a moiety for anchoring tags and/or drug molecules. Conventional methods of delivering immunosuppressant drugs often require a high dose of drugs to ensure therapeutic effects, but this can lead to toxic side effects. In this study, we used silica-coated iron oxide nanoparticles (IOSs) for a drug delivery application in which the nanoparticles carry the minimum amount of drug required to be effective to the target cells. IOSs could be loaded with water-insoluble immunosuppressive drug molecules (MPA: mycophenolic acid) and be used as a contrast agent for MRI. We characterized the IOSs for their physicochemical properties and found their average hydrodynamic diameter and core size to be 40.5 nm and 5 nm, respectively. Following the introduction of MPA-loaded IOSs (IOS/M), we evaluated the secretion dynamics of cytokines from peripheral blood mononuclear cells stimulated with phytohemagglutinin (PHA). The results showed that IOS/M effectively inhibited the secretion of the cytokines interleukin-2 and tumor necrosis factor α, with a minimal concentration of MPA. In conclusion, IOS/M may have potential applications in both efficient drug delivery and MRI.

1. Introduction

Nanoparticles haven been actively applied to drug delivery and molecular imaging studies by their encapsulation or surface modification with therapeutics [1,2]. Cell targeting by nanoparticles is another active research field applying nanotechnology to medicine [3]. Iron oxide nanoparticles (IONs) are one of the first nanomaterials to be approved for clinical application, mainly owing to their low toxicity and because they retain their biocompatibility when metabolized in the body [4,5]. Targeted drug delivery is possible with magnetic labeling of drugs on IONs, which can be guided by external magnetic fields to deliver the IONs to the objective sites and trigger the tissue-specific release of drugs [6]. The physicochemical properties of IONs determine their colloidal stability, drug release behavior, and magnetic resonance intensity, which affect their biomedical applications [7–9]. The current drug delivery approaches employing IONs involve the introduction of polymeric chains (*e.g.*, poly(ethylene glycol) (PEG), dextran, chitosan, poly(ethylenimine) (PEI), or copolymers, such as PEI–PEG–chitosan) to the Fe_3O_4 magnetic core in order to improve the stability, solubility, and drug loading capacity [10,11]. Another approach is to incorporate magnetic nanoparticles and drug molecules into liposomes for drug delivery [12,13]. Despite the complicated nature of the surface modification and incorporation methods, they are being increasingly used [8,14]. In this work, a silica coating on a Fe_3O_4 magnetic core improves the solubility of nanoparticles with hydrophobic drug molecules attached on the surface by hydrophobic or electrostatic interactions.

Mycophenolic acid (MPA) is the active ingredient of the immunosuppressant mycophenolate mofetil, which is widely used in organ transplants to prevent acute rejection or in autoimmune diseases to reduce autoreactive immune responses. MPA inhibits the proliferation of T- and B-lymphocytes by restricting inosine monophosphate dehydrogenase, which is involved in the biosyn-

* Corresponding author at: School of Integrative Engineering, Chung-Ang University, Seoul 156-756, Republic of Korea. Fax: +82 2 813 8159.
E-mail address: jonghoonc@gmail.com (J. Choi).

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thesis of guanine nucleotides required for lymphocyte growth [15]. Solid organ transplantation is the only hope for end-stage organ disease patients, and appropriate treatment of patients with immunosuppressant drugs reduces the probability of acute rejection and increases the percentage of survival rate [16]. Excessive use of MPA, however, leads to dose-dependent side effects, for example, hematologic and/or gastrointestinal effects [17]. In addition, MPA is only applicable to solid organ transplantation and it has been reported that the immunomodulation by MPA may result in secondary infection [18]. Therefore, it is essential to have a proper carrier method to deliver the appropriate amount of MPA to the required location.

Here, we report an efficient approach to deliver MPA to human immune cells with concurrent MRI. MPA is bound to silica-coated iron oxide nanoparticles (IOSs) and delivered to peripheral blood mononuclear cells (PBMCs) by a phagocytosis. During phagocytosis, the cell surface is invaginated and nanoparticles would be transported into the cell by phagosomes [19]. Nanoparticles could also be uptaken by human macrophages through various pathways such as macropinocytosis, clathrin mediated endocytic, caveolae mediated endocytic, and/or scavenger receptor mediated endocytic pathways [20,21]. Although it is not clear to determine which pathway is mainly occurred for IOS/M entering into PBMCs, we have confirmed that internalized IOSs were positioning in the cytoplasm of cells (Supporting Fig. 1). According to previous studies, silica coated magnetic nanoparticles could be more uptaken by endocytosis than dextran coated magnetic nanoparticles were [22]. The immunosuppressive effects after the successful delivery of MPA were probed. By using MRI and fluorescence imaging, we confirmed that MPA-loaded IOSs (IOS/M) were taken up by PBMCs. Human PBMCs were stimulated by phytohaemagglutinin (PHA) and showed increased release of cytokines while IOS/M delivered to the T cells reduced the production of cytokines.

The reported approach of delivering immunosuppressive drugs using IONs may have potential in controlling drug efficacy and limiting the side effects from dosing high levels of MPA.

2. Material and methods

2.1. Preparation of IONs and IOSs

Magnetite nanoparticles (NPs; 5 nm) stabilized with oleic acid were synthesized by the thermal decomposition of an iron–oleate complex [23]. The resulting magnetite NPs were coated with silica shells using polyoxyethylene(5)-nonylphenyl ether (Igepal CO-520) as a surfactant and tetraethyl orthosilicate (TEOS) as a silica precursor[24]. Typically, 0.23 g of Igepal CO-520 was dispersed in cyclohexane (4.8 mL) by sonication, followed by the addition of 0.2 mL of magnetite NPs in cyclohexane (150 mg/mL). Ammonium hydroxide solution (50 μL) was then added to the mixture, followed by vigorous stirring to obtain a reverse microemulsion. After 1 h of stirring, 50 μL of TEOS was added and stirred for 24 h. The resulting silica-coated magnetite NPs were washed with ethanol three times and redispersed in 20 mL of ethanol.

2.2. Characterization of IOSs

The morphology, size, and size distribution of the silica-coated magnetite NPs were studied by using a transmission electron microscope (JEOL-2010 with 200 kV accelerated voltage). Samples were prepared by casting a drop of nanoparticle dispersion onto a carbon-coated copper grid.

The elemental (Fe ion) concentration was determined using an inductively coupled plasma atomic emission spectrometer (ICP-AES; ICPS-1000IV, Shimadzu, Japan). The room-temperature magnetization curves were obtained by using a Quantum Design PPMS-9 magnetometer. The IOSs' size distribution and zeta potential were analyzed with a dynamic light scattering-based, sub-micrometer particle analyzer (Zetasizer, Malvern, UK) and transmission electron microscopy (TEM) image analysis.

2.3. Preparation of IOS/M

MPA (Sigma Aldrich) was dissolved in dimethyl sulfoxide (DMSO), diluted to a stock concentration of 1 mg/mL and stored at −20 °C. IOS (1 mg/mL) was mixed with the MPA stock solution (1 mg/mL in DMSO) and rotated for 12 h. Centrifugation at 12000 × *g* for 5 minutes separated unreacted MPA. After repeated washing by adding 1 mL deionized water, the product was finally re-suspended in Phosphate buffed saline (PBS pH 7.4). IOS/M nanoparticles in PBS were then characterized for their physical and chemical properties.

2.4. MPA release profiling in aqueous solution

The UV absorbance spectra of 200 μL aliquots of IOS/M nanoparticles were recorded using a Synergy Mx (Biotek Inc., USA). Sustained release property of MPA molecules from IOS/M was profiled by measuring the absorbance spectra of the sample solution taken from the IOS/M suspension at different time points. MPA (1 mg/mL) and IOS (1 mg/mL) were mixed in DMSO for 12 h following the separation of IOS/M-bound products by ultracentrifugation (15,000 × *g*, 20 min). Every 3 hours, 200 μL of the sample was taken for the measurement. The measured samples were replaced with the same amount of DI water added to the solution. The integration of the absorbance spectra produced the total amount of released MPA. The absorption spectra of MPA, IOS/M and IOSs were measured and the area between 280 and 300 nm was integrated for each sample.

2.5. Cell viability assay

PBMCs (CTL, USA) were thawed and then cultured for 16 h before the experiment. PBMCs (1×10^6 cells) were plated in each well in 12-well plates and then treated with different concentrations of IOSs. After 24 h of culture in RPMI 1640 with 10% fetal bovine serum, 1% penicillin, and streptomycin at 5% CO_2, the numbers of live and dead cells were counted by Trypan blue dye staining in a JuLI BR cell counter (Invitrogen).

2.6. MR measurement of IOS/M loaded cells

PBMCs loaded with IOS/M were fixed in 5% agarose gel for MRI. MRI experiments were performed on a 3.0 T clinical MR imaging instrument (GE Signa 3.0 T). The pulse sequence used was a T2-weighted multi-echo spoiled gradient echo (SPGR) sequence. The obtained MR images of cells were processed to calculate the amount of IOS/M contained in the cells.

2.7. Cytokine profiling

The release of human interleukin-2 (IL-2) and tumor necrosis factor α (TNF-α) was assayed from the culture supernatants by ELISA (all reagents were obtained from BioLegend Inc.). Thawed PBMCs were kept in culture for 16 h and stabilized before they were introduced into 24-well plates at a concentration of 2×10^5 cells/well. The diluted MPA samples (0, 0.001, 0.01, 0.1, 1, and 10 μg/mL) were added to the wells and cytokine secretion was measured after 24 h of incubation. The highest concentration of IOS/M that does not show cytotoxicity was chosen and added to PBMCs stimulated with phytohaemagglutinin (PHA, 5 μg/mL,