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On the chemical synthesis and drug delivery response of folate receptor-activated, polyethylene glycol-functionalized magnetite nanoparticles

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Abstract

We describe here the chemical synthesis and in vitro drug delivery response of polyethylene glycol (PEG)-functionalized magnetite (Fe_3O_4) nanoparticles, which were activated with a stable ligand, folic acid, and conjugated with an anticancer drug, doxorubicin. The functionalization and conjugation steps in the chemical synthesis were confirmed using Fourier transform infrared spectroscopy. The drug-release behavior of PEG-functionalized and folic acid–doxorubicin-conjugated magnetic nanoparticles was characterized by two stages involving an initial rapid release, followed by a controlled release.

Keywords: Drug delivery; Folic acid receptor; Functionalization; Magnetite nanoparticles

1. Introduction

The unhindered spread of malignant cells in the human system is a matter of serious concern. The routes that are generally adopted to target the cancer cells include surgical resection, irradiation and chemotherapy. The selection of the approach, however, depends on whether the tumor is at an early or advanced stage. New therapeutic drugs are being developed [1], and a significant effort is being devoted to improving the non-invasive methods. The non-invasive methods that have received considerable attention include hyperthermia and photodynamic therapy because of the advances that have been made in the chemistry of photosensitizers [2], and feasibility of hyperthermia via the use of AC magnetic field-induced excitation of superparamagnetic nanoparticles [3].

The basic challenge in drug delivery is the transfer of drug agents to the targeted site at the appropriate time [4]. Orally administered anticancer drugs or injections suffer from the drawback of limited control on the rate of drug release in addition to harmful side effects and toxicity. In the case of therapeutic levels extending over longer periods of time, the preferred regime is an initially high release of drug, followed by a gradual decrease with time. The controlled delivery of a drug is an appropriate route to eliminate the above drawbacks and allows for continuous release of the drug. In controlled delivery, the drug delivery may commence with first-order kinetics to an optimum and effective drug concentration to the targeted region, followed by zero-order kinetics. It is possible to accomplish this by conjugating the drug to a polymer, thereby allowing the drug to be released in a pre-designed manner. However, it is important that the polymer is biodegradable and can be eliminated by the physiological system without leaving

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residues that may accumulate in the cell compartments such as liposomes or tissues from the phagocytosis system [5,6]. In addition, the use of polymeric nanoparticles in vivo requires them to be hydrophilic with a pH close to physiological (~7). Amorphous polymers are not considered suitable because they are vulnerable to degradation because of their high accessibility to water [7]. Furthermore, the high-molecular-weight, long-chain polymers require a number of chemical bonds to be broken for reduction to low-molecular-weight oligomers or monomers, a necessary step in the biodegradation process.

Magnetic field-induced, targeted drug delivery is currently attracting significant interest [8,9]. The conjugation of a drug to magnetic nanoparticles encapsulated with polymer is a viable method for controlled delivery of a drug to specific sites. Other potential applications of magnetic nanoparticles in biomedical and diagnostic applications include magnetic resonance imaging, hyperthermia, tumor treatment, separation of DNA, and site-specific gene and drug delivery [10–18]. An important advantage of magnetic nanoparticles is the use of a localized magnetic field gradient to attract the particles to the desired site, hold them at the desired location until the therapy is completed, followed by removal. This approach necessitates that the magnetic nanoparticles have adequate magnetic strength, biocompatibility and interactive functions on the surface [19]. In view of the above, the application of magnetic nanoparticles in diagnostics, magnetic imaging, targeted delivery and cancer treatment is the subject of much attention.

In the aforementioned methodology, the particles are injected intravenously and blood circulation transports these particles to the region of interest for treatment. However, in this approach, it is important to consider the following aspects. First, the particles should not aggregate but rather be individually dispersed; if they are aggregated, then their spread or distribution is hindered and rapid removal from the circulation is promoted. Second, when the particles are injected into the bloodstream they are rapidly coated with circulation components, such as plasma proteins. This adsorption of proteins at the particle surface is referred as opsonization [20]. Thus, it is necessary to render the particles hydrophilic to inhibit the plasma protein coating process [21].

The targeted delivery is a viable route to increase the effective use of a drug and minimize undesirable side effects and toxicity [22,23]. Intracellular uptake of nanoparticles into cells can be accomplished by liquid phase endocytosis [24], receptor-mediated endocytosis [25] or phagocytosis [26]. A logical method to promote internalization of nanoparticles is to modify their surface with a ligand such as folic acid, which can be efficiently taken up by the cells through receptor-mediated endocytosis [26]. The folate receptor targets are considered appropriate for tumor-selective drug delivery for a number of reasons. Folate is required to replicate DNA and synthesize RNA and has the advantage of not causing changes in DNA that can lead to cancer. More importantly, folic acid is stable, poorly immunogenic and has the ability to preferentially target cancer cells because the folate receptor is frequently overexpressed on the surface of cancer cells [27]. In general, folate receptor is a glycosylphosphatidylinositol-anchored, high-affinity, folate-binding protein that is overexpressed in different kinds of tumors. It functions by stopping folate from nourishing the rapidly dividing tumor cells [28–30]. The reason is that the folate receptor provides preferential sites that differentiate tumor cells from normal cells. The conjugates of folate are suitable for targeted delivery because folate lacks immunotoxocity and also because of its role in receptor-mediated endocytosis. The process involves entry of a folate–receptor complex into the cell as a group, where it is fused with a lysosome [31,32]. Subsequently, the folate is released from the lysosome and the receptor is recycled back to the surface of the cell to capture additional folate. Drugs and drug carriers that have been investigated as folate conjugates are protein toxins, liposomes and plasmid DNA complexes [31,32]. However, in spite of the well-known ability of folate receptor to assist in internalization, studies on the drug delivery response using folic acid-activated, polymer-coated magnetic nanoparticles have not been adequately researched. In a very recent study, Sun et al. reported an important study on the development and in vitro examination of folic acid–polyethylene glycol (PEG)-conjugated superparamagnetic nanoparticles that served as a targeted magnetic resonance imaging contrast agent for detection of cancer cells overexpressing the folate receptor [33]. Their work underscores the relevance of further examining folic acid–PEG-coated magnetic nanoparticles for targeted drug delivery.

In previous papers, we described the magnetic behavior and appropriateness of magnetic nanoparticles with narrow size distribution (5–8 nm) for biomedical applications with regard to biocompatibility [34]. In the present work, we discuss the chemical synthesis and drug release response of PEG-functionalized magnetite (Fe_3O_4) nanoparticles that were tethered to folic acid and conjugated with the anti-cancer drug doxorubicin.

2. Experimental

2.1. Chemical synthesis of magnetite nanoparticles

Magnetite (Fe_3O_4) nanoparticles with average particle size of ~7 nm were prepared by a high-temperature decomposition process [35,36]. First, 20 ml of biphenyl ether, 0.71 g (2 mmol) of iron(III) acetylacetonate, 2.25 g (10 mmol) of 1,2-dodecanediol, 2.12 ml (6 mmol) of oleic acid, and 2.19 ml (6 mmol) of oleylamine were thoroughly mixed by magnetic stirring in a 150 ml three-necked flask. The solution was heated to 200 °C in a nitrogen atmosphere for 2 h. After 2 h, the nitrogen flow was stopped and the solution was heated at ~260 °C for 1 h to reflux. During this process, the initial brown-black color of the solution changed to dark black, implying the synthesis of the magnetite nanoparticles. Subsequently, the resultant

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