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Magnetically triggered nanovehicles for controlled drug release as a colorectal cancer therapy

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ABSTRACT

Magnetic silica core/shell nanovehicles presenting atherosclerotic plaque-specific peptide-1 (AP-1) as a targeting ligand (MPVA-AP1 nanovehicles) have been prepared through a double-emulsion method and surface modification. Amphiphilic poly(vinyl alcohol) was introduced as a polymer binder to encapsulate various drug molecules (hydrophobic, hydrophilic, polymeric) and magnetic iron oxide (Fe_3O_4) nanoparticles. Under a high-frequency magnetic field, magnetic carriers (diameter: ca. 50 nm) incorporating the anti-cancer drug doxorubicin collapsed, releasing approximately 80% of the drug payload, due to the heat generated by the rapidly rotating Fe_3O_4 nanoparticles, thereby realizing rapid and accurate controlled drug release. Simultaneously, the magnetic Fe_3O_4 themselves could also kill the tumor cells through a hyperthermia effect (inductive heating). Unlike their ungrafted congeners (MPVA nanovehicles), the AP1-grafted nanovehicles bound efficiently to colorectal cancer cells (CT26-IL4Rα), thereby displaying tumor-cell selectivity. The combination of remote control, targeted dosing, drug-loading flexibility, and thermotherapy and chemotherapy suggests that magnetic nanovehicles such as MPVA-AP1 have great potential for application in cancer therapy.

1. Introduction

The development of nanovehicles capable of multifunctional tumor therapies has received much attention in biomaterial research because of their potential use in efficient cancer treatment [1–4]. For example, polymeric nanovehicles are attractive drug carriers because of their small size [5,6] and highly flexible structural modification with biological cell-binding groups (e.g., antibodies, ligands, peptides) capable of enhancing the targeting effect [7]. Furthermore, polymeric nanovehicles can also display long-lasting circulation characteristics and high-accumulation properties in tumor cells, owing to inefficient drainage by the lymph system—the so-called enhanced permeation and retention (EPR) effect [8–10].

Many reports have also been published regarding stimuli-response nanovehicles, where the temperature [11–13], pH [14–16], ionic strength [17], and magnetic field [18–23] can be used as driving forces for drug delivery and controlled release. Among these stimuli, magnetic fields, a noncontact force, have been used widely in diagnostic and therapeutic applications because they allows remote treatment through delivery followed by magnetically triggered release [24]. Iron oxide (Fe_3O_4) is most commonly employed as the magnetic trigger because it is amenable to surface modification, self-assembly, and nanovehicle encapsulation [25–28]. In addition to remote-controlled release, magnetic Fe_3O_4 can also facilitate simultaneous magnetic resonance imaging of bio-

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logical responses to anti-cancer therapy [29], as well as generate heat, through its magnetic hyperthermic capability, to compromise tumor cells [30]. The synergistic combination of chemotherapy and hyperthermia appears to have great potential as a cancer therapy, with tissue deoxygenation and cell death occurring at temperatures above 42 °C [31].

The application of traditional drug carriers in nanovehicles remains hindered, however, by low drug loading contents (DLCs), poor drug-loading flexibility, rapid leakage, poor release profiles, and low stabilities [24,32]. Although various nanovehicles have been developed to overcome these obstacles (e.g., cross-linked nanovehicles providing stability without leakage; large-volume liposome-based nanovehicles that increase DLCs; stimuli-responsive vehicles for controlled release), relatively few investigations have been conducted into accentuating the drug-loading flexibility. The ability to encapsulate a variety of drugs is desirable because such nanovehicles might be practically applicable to a wider range of illnesses, rather than specific ones. In this study, we developed a smart nanovehicle that can incorporate magnetic Fe_3O_4 and various kinds of drugs in a polymeric nanoparticle.

Although the EPR effect can improve the therapeutic efficiency of nanoparticles, a practical therapy for killing tumors would involve targeted cellular delivery through enhanced selectivity toward tumor cells over normal tissue [33]. Various human solid tumor cell lines overexpress high-affinity interleukin-4 receptors (IL-4Rα) [34–36]. Thus, targeted delivery might be feasible by presenting a selective ligand for IL-4Rα from the surface of the nanovehicle. Therefore, we modified our nanovehicles with atherosclerotic plaque-specific peptide-1 (AP-1) [37], to enhance targeting through greater binding selectivity to the IL-4Rα receptors.

In this study, we used a double-emulsion method [38–40] with amphiphilic poly(vinyl alcohol) (PVA) as a polymer binder to encapsulate drug molecules of various hydrophilicities. We also employed silica nanoshells to minimize leakage in the absence of the extrinsic stimulus and to provide surface reaction sites for modification with the targeting (AP-1) ligands [41–43]. The targeting effect could be monitored using a differential interference contrast microscope. An external high-frequency magnetic stimulus induced controlled release, allowing the accumulation release percentages to be recorded through UV–vis spectroscopy.

2. Experimental

2.1. Materials

Benzyl ether (99%), 1,2-hexadecanediol (97%), oleic acid (90%), oleylamine (>70%), iron(III) acetylacetonate [$Fe(acac)_3$], absolute EtOH (99.5%), 1-ethyl-3-(dimethylaminopropyl) carbodiimide hydrochloride (EDC) were purchased from Aldrich. PVA (M_w: 16,000, 47,000, 61,000) was obtained from Fluka. Tetraethyl orthosilicate (TEOS) and 3-aminopropyltrimethoxysilane (APTMS) were purchased from Sigma. Doxorubicin hydrochloride (DOX), vitamin B12, curcumin, cytochrome C, and myoglobin (all from Aldrich) were used as model drugs. AP-1 was obtained from Biotools. All other chemicals and solvents were purchased from Sigma–Aldrich, Acros, or Fluka and used as received, except as noted.

2.2. Ferrite fluid

Fe_3O_4 was synthesized reported previously [44]. $Fe(acac)_3$ (0.706 g) was mixed with 1,2-hexaecanediol (2.51 g), lauric acid (1.20 g), and dodecylamine (1.12 g) in benzyl ether (20 mL) and heated under reflux at 200 °C for 30 min and then at 300 °C for 1 h under N_2. After cooling to room temperature, the black powder was collected by centrifugation (6500 rpm, 5 min) and washed three times with excess EtOH.

2.3. MPVA nanovehicles: double-emulsion method

The magnetic PVA-based nanovehicles (MPVA) were prepared using water-in-oil-in-water ($W_1/O_1/W_2$) processes. The water phases (W_1, W_2) contained 2% aqueous PVA (10 mL) as a polymer binder; the oil phase (O_1) was a dispersion of Fe_3O_4 nanoparticles (60 mg) in $CHCl_3$ (4 mL). W_1 (1.6 mL) was mixed with O_1 through ultrasonication; the mixture was added into W_2 with ultrasonication to obtain the double-emulsion nanovehicles. The organic solvent was evaporated through heating and the residue was washed with deionized water and collected through centrifugation (6500 rpm). The resulting magnetic nanovehicles having a uniform and spherical configuration were re-dispersed in DI water.

2.4. AP-1 grafting

Silica shells were coated on the MPVA nanoparticles using a modification of the Stöber method [45]. MPVA (5 mg) was dispersed in 99.5% EtOH (4 mL); TEOS (40 μL) and APTES (40 μL) were added slowly and separately to the solution, which was then stirred for 30 min. 35% NH_4OH (100 μL) was added and then the mixture was stirred for 12 h. After hydrolysis and condensation, the silica shell-coated nanoparticles (MPVA-APTES) were obtained. AP-1 solution (0.1 wt%, 20 μL) was added and then the mixture was incubated at 4 °C for 2 h; EDC solution (0.1 wt%, 50 μL) was added and then the mixture was stirred for 4 h. The AP-1-grafted nanovehicles (MPVA-AP1) were collected and washed with excess DI water through centrifugation (6500 rpm).

2.5. Drug loading

Amphiphilic drug-loaded nanovehicles were obtained by modifying the procedure described above. A model drug (10 mg) was dissolved in the appropriate solvent: hydrophilic drugs (e.g., DOX, vitamin B12) were dissolved in W_1 (PVA solution, 8 mL), while the hydrophobic drug curcumin was dissolved in O_1 ($CHCl_3$, 20 mL). This phase was then used to prepare the double-emulsion and the AP-1-grafted nanovehicles using the aforementioned protocols. The drug-loaded nanovehicles were gathered through centrifugation (6000 rpm, 5 min). The concentration of the free drug was measured using UV–vis spectroscopy, allowing quantification of the encapsulated model drug in the nanovehicles. The model drugs had the following maximum absorption wavelengths: vitamin B12 (316 nm); DOX (482 nm); curcumin (549 nm); cytochrome C (550 nm); myoglobin (409 nm).

2.6. Stimuli-triggered drug delivery

In vitro drug release from the magnetic nanovehicles incorporating the various drugs was evaluated after radio heating, generated by a high-frequency magnetic field (HFMF), from an Induced Heating Machine (LT-15-80) operated at 15 kV and 50–100 kHz. At specific time intervals during the magnetic field-triggered release, aliquots of the solution were withdrawn and the concentration of released drugs measured using UV–vis spectroscopy. The temperature was also recorded to determine the cell-killing effect arising from the hyperthermic therapy.

2.7. Cell culture

Mouse colon carcinoma cell line (CT26) was obtained from the Bioresource Collection and Research Centre (BCRC, Taiwan). Con-