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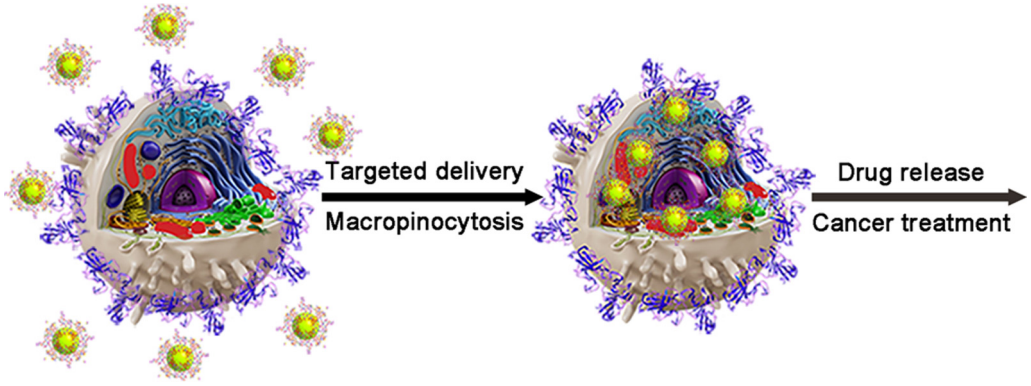
Targeted delivery and pH-responsive release of doxorubicin to cancer cells using calcium carbonate/hyaluronate/glutamate mesoporous hollow spheres

Yuming Guo [a,b,*], Han Li [a], Weike Shi [a], Jie Zhang [a], Jing Feng [a], Xiaoli Yang [a], Kui Wang [a], Hua Zhang [a], Lin Yang [a,b,*]

[a] Collaborative Innovation Center of Henan Province for Green Manufacturing of Fine Chemicals, Key Laboratory of Green Chemical Media and Reactions, Ministry of Education, Henan Normal University, Xinxiang, Henan 453007, PR China
[b] Henan Key Laboratory of Green Chemical Media and Reactions, School of Chemistry and Chemical Engineering, Henan Normal University, Xinxiang, Henan 453007, PR China

GRAPHICAL ABSTRACT

Herein, calcium carbonate/hyaluronate/glutamate mesoporous hollow spheres were prepared and used for targeted delivery and pH-sensitive release of anticancer drugs to treat human cancers.



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ABSTRACT

Currently, the efficacies of the existing anticancer drugs used in chemotherapy are still unsatisfactory. Therefore, drug delivery system has received considerable research interest. In the present study, calcium carbonate/hyaluronate/glutamate mesoporous hollow spheres are prepared through a facile method. The results indicate that the mesoporous hollow spheres can efficiently load the anticancer drug doxorubicin. Through the specific binding of hyaluronate on hollow spheres with CD44 receptors overexpressed on cancer cells, the drug-loaded hollow spheres can be specifically delivered to target cancer cells. Owing to the gradually dissolution of calcium carbonate in the weak acidic microenvironment of cancer cells, the loaded doxorubicin can be released over the period of 14 days with pH-responsive and sustained manner to specifically and significantly treat cancers. Through loaded onto the hollow spheres, the IC_{50} value of doxorubicin for HeLa cancer cells is 0.0113 μg/mL, much lower than that of the free doxorubicin (0.0801 μg/mL). However, the IC_{50} value of doxorubicin for V79-4 cells is 0.2032 μg/mL, obviously

* Corresponding authors at: Henan Key Laboratory of Green Chemical Media and Reactions, School of Chemistry and Chemical Engineering, Henan Normal University, Xinxiang, Henan 453007, PR China.
E-mail addresses: guoyuming@htu.edu.cn (Y. Guo), yanglin1819@163.com (L. Yang).

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higher than that of the free DOX (0.1396 μg/mL). The specificity of the doxorubicin between normal and cancer cells can be enhanced about 10-fold. The current study suggests the possible application of pH-responsive inorganic carriers for efficiently treatment of human cancers.

1. Introduction

Currently, on account of the unfavorable pharmacokinetics, poor biodistribution and selectivity, the treatment efficacies of the existing chemotherapeutic drugs still do not meet the clinical demands [1–4]. Because of the significant enhancement of treatment efficacy and reduction of the systemic side effects of the free drugs by altering the pharmacokinetics and biodistribution, drug delivery system (DDS) has attracted considerable research interest [5–8]. Practically, an ideal DDS should eliminate the tissue damage on accidental extravasation, protect the drugs from premature degradation, reduce the side effects on nontarget tissues, and increase the local concentrations of the drugs [9]. In addition, the facile preparation and formulation, high drug loading capacity, and specific delivery to target cancer cells are critical for their clinical application [10,11]. Moreover, it is also important to release the drug in response to stimuli associated with the specific microenvironmental changes of cancer cells with sustained manner [12,13]. Previously, many studied DDS are constructed using polymers [14,15], lipids [16], and surfactants [17]. Recently, inorganic materials were considered as promising DDS candidates because of the simple preparation and reduced involvement of toxic agents, such as mesoporous silica [18–20], iron oxide [21], and hydroxide [22]. Similar to these inorganic drug carriers, $CaCO_3$ also might be an ideal DDS candidate because of the good biocompatibility and biodegradability [23–25].

Herein, $CaCO_3$/hyaluronate/glutamate hybrid mesoporous hollow spheres ($CaCO_3$/HA/Glu MHSs) were prepared through a facile strategy under mild conditions. The results indicate the simultaneous presence of two types of mesopores in MHSs. This interesting property contributes to the efficient loading and sustained release of anticancer drug doxorubicin (DOX). Furthermore, because of the inherently good biocompatibility, pH sensitivity, and biodegradability of $CaCO_3$, $CaCO_3$/HA/Glu MHSs can release the DOX in controlled and sustained manner in response to the weak acidic microenvironment of cancer cells. More importantly, because of the highly specific binding of HA with CD44 receptor overexpressed on cancer cells [26,27], $CaCO_3$/HA/Glu/DOX could be specifically delivered to target cancer cells. Using $CaCO_3$/HA/Glu MHSs as drug carrier, the specificity of DOX between normal cells and cancer cells is enhanced about 10-fold. The specificity of the DOX and the total drug release period are both much better than our previous results [28,29]. Together with our previous studies [23,25,28–30], the present study further demonstrates and expands the applications of pH-responsive inorganic materials as drug carriers to efficiently treat cancers.

2. Experimental

2.1. Chemicals

Calcium chloride ($CaCl_2$, ACS grade), sodium carbonate (Na_2CO_3, ACS grade), sodium hyaluronate (BioReagent grade), sodium bicarbonate (ACS grade), DOX (98%), sodium glutamate (BioReagent grade), and 3-(4,5-dimethylthiazol-2-yl)-2,5,diphenyl tetrazolium bromide (MTT, BioReagent grade) were purchased from Sigma-Aldrich CO. Eagle's minimum essential medium (EMEM), fetal bovine serum (FBS), and antibiotic-antimycotic solution (100 X) were purchased from Thermo Fisher Scientific Inc. All chemicals were used as received without further purification.

2.2. Preparation of $CaCO_3$/HA/Glu MHSs

The $CaCO_3$/HA/Glu MHSs were prepared as follows. Briefly, $CaCl_2$ aqueous solution (10.0 mL, 5 mM) was added slowly dropwise into 30 mL of mixed aqueous solution of Glu (5 mM) and HA (0.05 g) and moderate stirred for 2 h at 25 °C. Then, the aqueous solution of Na_2CO_3 (5 mM, 10 mL) was added and reacted for 24 h at 25 °C. Finally, the product was collected by centrifugation and rinsed with DD water and absolute ethanol for several times. The as-prepared product was dried under vacuum and denoted as $CaCO_3$/HA/Glu MHSs. For comparison, the experiments were also performed in the sole presence of HA or Glu or in the absence of HA and Glu under nearly identical conditions as the typical experiment.

2.3. Characterization

In the current study, the size and morphology of the samples were determined by scanning electron microscopy (SEM, JSM-6390LV, JEOL) and high-resolution transmission electron microscopy (HR-TEM, JEM-2100, JEOL). The crystal phases of the samples were determined by powder X-ray diffraction (XRD) using a D8ADVANCE X-ray diffractometer (Bruker axs Com., Germany) with graphite monochromatized Cu Kα radiation (λ = 0.15406 nm). The XRD patterns of the samples were recorded in the 2θ range of 20-70°. The organic contents of the samples were determined by thermogravimetry-differential scanning calorimetry (TG-DSC, STA 449C, NETZSCH) analysis in the range of 25-900 °C with a linear heating rate of 10 °C/min. The specific surface area and pore size distribution were analyzed through the Bru nauer–Emmett–Teller (BET) determination at liquid nitrogen temperature using N_2 as an adsorbent (Gemini 2380, Micromeritics).

2.4. DOX loading and the incorporation efficiency

$CaCO_3$/HA/Glu MHSs (7 mg) were mixed with DOX aqueous solution (7 mL, 100 μg/mL) and shaken in an orbital shaker for 24 h at 25 °C to load the DOX. Subsequently, the mixture was centrifuged and the precipitate was rinsed with ultrapure water until the supernatant changed to colorless. The obtained precipitate was dried and denoted as $CaCO_3$/HA/Glu/DOX. All the supernatants were collected together. To determine the loading efficiency, the amounts of the free DOX in the supernatants were determined by UV–Vis absorbance. The incorporation efficiency is reported using loading content (wt%) and entrapment (wt%) calculated by Equation (1) and (2), respectively. The reported data are the mean values of triplicate determinations ± standard deviation (SD).

$$Loading\ content(\%w/w) = \frac{mass\ of\ DOX\ in\ CaCO_3\ /HA/Glu/DOX \times 100}{mass\ of\ CaCO_3\ /HA/Glu/DOX} \quad (1)$$

$$Entrapment(\%w/w) = \frac{mass\ of\ DOX\ in\ CaCO_3\ /HA/Glu/DOX \times 100}{mass\ of\ DOX\ used\ in\ formulation} \quad (2)$$