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Synthesis and characterization of aminotetrazole-functionalized magnetic chitosan nanocomposite as a novel nanocarrier for targeted gene delivery

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ABSTRACT

In the present study, Fe_3O_4/chitosan biopolymer grafted to a novel organosilane modified 5-amino-1*H*-tetrazole, a kind of drug intermediate, was successfully synthesized by chemical modification technique and evaluated as a high potential carrier of gene delivery. The loading capacity was evaluated, and in vitro release of nanocarrier was assessed using the dialysis method. The transfection efficiency of plasmid was optimal at an N/P ratio of 3. The chemically modified chitosan showed no inherent toxicity toward the cells. The synthesized nanocarrier had enhanced release of the plasmid at physiological pH 7.4. The N-functionalized magnetic chitosan nanocarrier demonstrated its efficacy in the enhancement of gene expression in the HECK-293T cell line. Therefore, the novel magnetic N-functionalized chitosan showed promise as a highly efficient gene carrier with potential applications in cancer therapy.

1. Introduction

Gene therapy refers to the transmission of a therapeutic gene into the targeted cells with consequent expression of the transgene. However, the unprotected DNA can be rapidly degraded and also, the negative charge of the naked DNA inhibit from its entering the cells which have the same charge [1]. Therefore, more attention is focused on the introducing of the novel gene carrier [2–6]. As a gene carrier, the conventional virus has fatal drawbacks of low transfection rate, cell toxicity [7], and unwanted immune response as well as oncogenic effects [1]. As a non-viral vector, chitosan (CS) consisted of repeating D-glucosamine and *N*-acetyl-D-glucosamine units, linked via (1–4) glycosidic bonds [8], is a naturally abundant and biodegradable linear biopolymer [9]. Chitosan has exhibited biocompatibility and low toxicity in experimental animals and humans [10] which could be able to provide strong binding with the plasmid to well protect DNA from nuclease degradation [11]. More, besides the chitosan being used for gene delivery, is used in tissue engineering and drug delivery [12–14]. So, chitosan and chemically modified chitosans are used as cationic polysaccharides for gene delivery to cancer cells with a large utility in controlled release and targeting studies of therapeutic molecules [15–17]. Nevertheless, chitosan with the pKa of 6.5 has a limited solubility at physiological pH with poor gene transfection efficiency [18] and rapid degradation [19]. Therefore, chemical modifications of chitosan structure have been won a worldwide attention to improving the stability and transfection efficiency [20–24]. The primary chitosan amino groups are responsible for properties such as controlled release, transfection, mucoadhesion, in situ gelation, permeation enhancement, and efflux pumps inhibitory properties [25]. In this regards, the primary hydroxyl and nucleophilic amino groups of the chitosan backbone are suitable sites for chemical modification [26,27] to overcome its limitation in controlled and targeted gene delivery. Herein, we report the synthesis and characterization of magnetic amino-functionalized chitosan (MAFCS) that can improve solubility, stability and binding affinity to the plasmid and biocompatibility of gene carrier. This nanocarrier was obtained by functionalizing of chitosan amino group with propyl-(1*H*-tetrazole-5-yl)amine-trimethoxysilane intermediate which synthesized via the reaction of 5-aminotetrazole with (3-chloropropyl) trimethoxysilane (Scheme 1), As well as the chitosan primary hydroxyl groups were modified by coating with Fe_3O_4 magnetic nanoparticles (MNPs) to improve transfection efficiency. Then, the potential of the desired nanocarrier was fully evaluated for using in cancer therapy through studies of its cytotoxicity, transfection efficiency and gene uptake in HEK-293T cell line. More, it was found that magnetic amino-functionalized chitosan nanoparticles had low cell toxicity and condensing DNA effectively with high transfection efficiency in the

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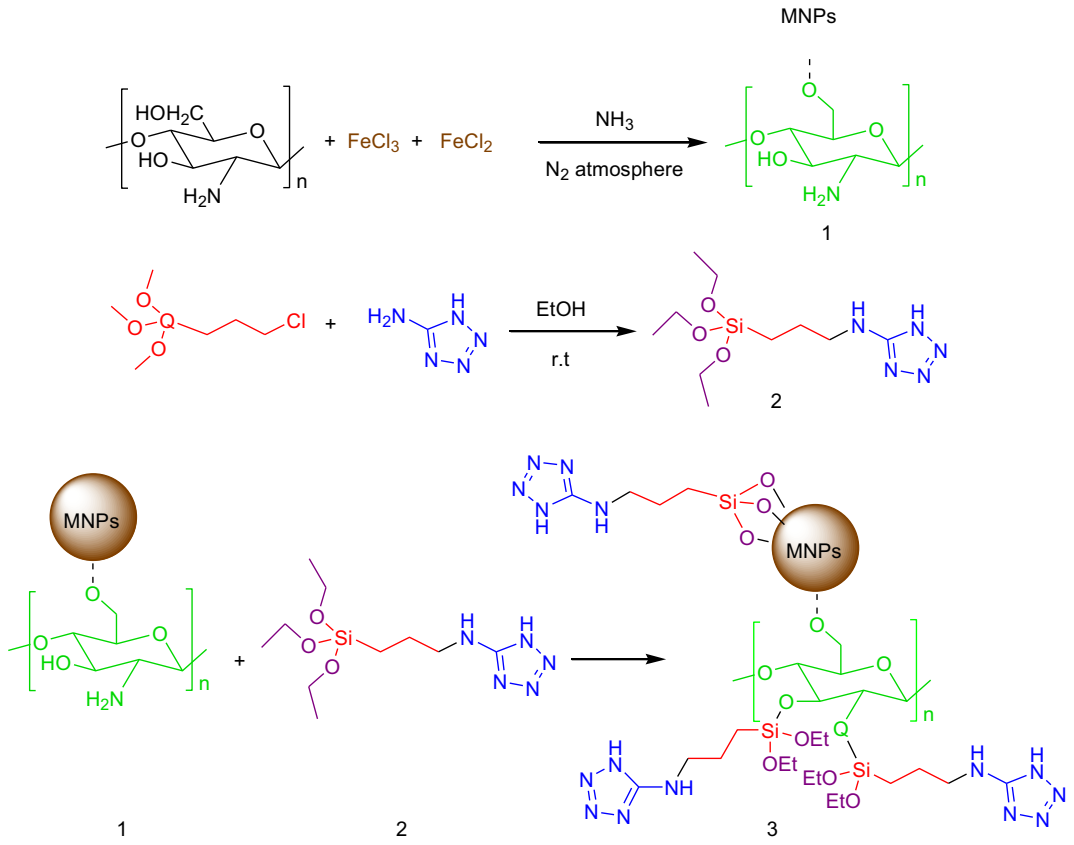

Scheme 1. Synthesis of MAFCS nanocarrier.

magnetic field.

2. Experimental section

2.1. Materials and instrument

Chitosan hydrochloride with medium MW was purchased from Merck. Another chemicals used in this work, were of analytical reagent grade, purchased from Merck and used without further purification. Double deionized water was used for all dilutions. NMR spectra were recorded at 500.133 (^{1}H) MHz on Bruker DRX-500Avance spectrometer at 500.133 MHz, respectively. IR spectra were obtained with MATSON 1000 FT-IR spectrophotometer. IR spectra were obtained with MATSON 1000 FT-IR spectrophotometer. X-ray diffraction (XRD) with an X-Pert Philips PW340/60 diffractometer (40 kV and 30 mA) and Cu K_α radiation (λ = 0.154 nm) was used to analyze the crystal structure of the carrier. The zeta potential, mean size and size distribution of the nanocarrier were measured by zetasizer nano ZS instrument (Malvern, England).The morphology of the cross-section of the film was examined by a field emission scanning electron microscope (FESEM) (Hitachi S4160) and transition electron microscopy (TEM) (Philips, CM 120). Sonication was performed by using a Hielscher ultrasonic instrument (model UP200S, 200 W, USA).The sample quantities were characterized by spectrophotometer (Cary 50, Varian). The AFM photos were prepared using Dme microscope with ds95-50 scanner (Denmark).

2.2. Synthesis of Fe_3O_4/chitosan magnetic nanoparticles(MNPs-CS)

Chitosan hydrochloride with medium weight was used for the synthesis of nanocarrier. To synthesize the chitosan coated magnetic iron oxide nanoparticles (MNPs-CS), 0.1 g of chitosan was dissolved in 50 ml of aqueous acetic acid solution (1.0%) by stirring for 30 min at 60 °C. Then, a 15 mL of aqueous solution of Fe (II) and Fe (III) salts at a 1:2 ratio was dropwise added to the chitosan solution under N_2 atmosphere and stirred vigorously for 1 h in 40 °C. At the following, 20 mL of NH_4OH (15% v/v) dropped wise added to the reaction mixture to obtain the pH of 10. The black MNPs-CS were rapidly precipitated. The reaction temperature was increased to 80 °C and proceeded for 30 min. At the end, the desired product was collected by external magnetic field, washed with distilled water, redispersed in water, sonicated for 30 min and kept in 4 °C for the next steps.

2.3. Synthesis of propyl-(1H-tetrazole-5-yl)amine-trimethoxysilane

2 mmol of 5-amino-1*H*-tetrazole (0.206 g) was added to a round flask bottom containing 30 mL of absolute EtOH as the solvent. The flask was stand in an ice batch and then 2 mmol of (3-chloropropyl) trimethoxysilane (0.368 mL) was dropwise added to the reaction mixture and stirred for 2 h. The completion of the reaction was confirmed by TLC. The product was obtained as soluble in EtOH.

2.4. Synthesis of magnetic amino functionalized chitosan (MAFCS) nanocarrier

The soluble intermediate that obtained from Section 2.3, was dropwise added to 50 ml of dispersed aqueous MNPs-CS for 30 min. The reaction temperature was increased to 50 °C. Afterward, 10 mL of aqueous solution of KCO_3 (0.1 g) was added to the mixture and stirred for 24 h. After cooling, the final brown colored product was centrifuged and washed two times with distilled water, redispersed in distilled water, sonicated for 30 min and saved at 4 °C for *biological tests*.