ACTA PHYSICO-CHIMICA SINICA
Volume 23, Issue 12, December 2007
Online English edition of the Chinese language journal

Available online at www.sciencedirect.com
ScienceDirect

Cite this article as: Acta Phys. -Chim. Sin., 2007, 23(12): 1857−1863.

ARTICLE

Preparation and Characterization of a Novel Magnetic Nano-Gene Vector

Lijuan Hao, Shuangyan Li, Lei Han, Jie Huang, Jin Chang*

Institute of Nanobiotechnology, School of Materials Science and Engineering, Tianjin University, Tianjin 300072, P. R. China

Abstract: In recent times, as nonviral gene vectors, chitosan and its derivations have attracted many researchers′ attentions. The preparation of a novel gene vector-magnetic nanoparticle coated with a novel polymer-lysine modified chitosan (CTS-lys) is described in this article. Initially, the correlation conditions of preparing CTS-lys were optimized, and it was demonstrated that lots of amines of chitosan were modified by lysine, which was indicated by infrared spectroscopy (IR) and proton nuclear magnetic resonance (^{1}H-NMR). Subsequently, through the coprecipitation method, the lysine modified chitosan magnetic nanospheres (CTS-lys-MNPs) were attained. The characterizations of nanospheres were measured by transmission electron microscopy (TEM), dynamic laser light scattering, vibrating samples magnetometer (VSM), and X-ray diffraction (XRD). The cytotoxicity of CTS-lys-MNPs was also investigated with U293 cells. The results indicated that the average size of nanospheres was about 100 nm, and they had narrower size distribution, good superparamagnetic property, and perfect crystallinity, and they also retained low toxicity. Additionally, the combination of DNA and CTS-lys-MNPs was observed *via* agarose gel electrophoresis, and the ability of complexes of CTS-lys-MNPs and DNA crossing blood-brain barrier (BBB) in rats was studied by single photon emission computed tomography (SPECT). The results showed that the gene vector was a superior material, which had the ability of targeting and avoiding the caption of BBB.

Key Words: Chitosan; Lysine; Magnetic nanospheres; Target; Gene vector; Blood-brain barrier

Gene therapy is promising for curing various inherited or acquired diseases. However, the lack of safe and efficient carriers of DNA is a main hurdle to reach the success of gene therapy[1]. Great concern toward the safety of viral vectors makes the nonviral ones much more attractive.

In the past decades, chitosan (CTS), a naturally occurring linear cationic polysaccharide, has been widely employed as a drug/gene delivery system for wound dressing, as an anticoagulant and a scaffold for tissue engineering, owing to its biocompatibility, biodegradability, and low toxicity[2]. But as chitosan is insoluble in neutral or biological solutions and readily precipitated within a few days, it is difficult for chitosan-based self-aggregates to be widely applied to drug/gene delivery systems[3,4]. Recently, water-soluble chitosan derivatives have been used to increase their stability and decrease cytotoxicity[5,6].

Additionally, it is well known that there has been a great interest in developing and testing iron oxide nanoparticles for tumor detection and therapy in the past two decades. In brain research, nanodispersed iron oxides have been used as carriers of diagnostic and therapeutic agents for mapping the blood-brain barrier disruption, to improve tumor detection and therapy[7−9].

Here we have designed and synthesized a novel water soluble polymer (CTS-lys) and used it as a coat, to prepare magnetic microspheres. The reason that we have chosen CTS-lys as the coating agent is because it is biocompatible, biodegradable, nontoxic, and water soluble. Moreover, it also has some unique antitumor and antibacterial bioactivities[10]. Another appealing characteristic of CTS-lys is that it has more

Received: July 30, 2007; Revised: September 3, 2007.
*Corresponding author. Email: jinchang@tju.edu.cn; Tel: +8622-27401821.
The project was supported by the National Natural Science Foundation of China (50373033), the Key Project Foundation from Tianjin Science and Technology Committee, China (05YFJZJC01001), and the International Cooperation Foundation from Tianjin, China (05YFGHHZ20070).

Chinese edition available online at www.whxb.pku.edu.cn

positive charges than many other chitosan derivatives, which can improve the capacity for carrying DNA.

1 Materials and methods

1.1 Materials

Iron (III) chloride hexahydrate ($FeCl_3·6H_2O$), iron (II) chloride tetrahydrate ($FeCl_2·4H_2O$), ammonium hydroxide (5 $mol·L^{-1}$), hydrochloric acid (HCl), and dimethylfomamide (DMF) were obtained from local suppliers. Deionized water was used in all the steps involved in the synthesis and formulation of magnetic nanoparticles. Chitosan was obtained from Zhejiang Yuhuan Chitin Company (China). Lysine and *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (EDC) were both obtained from Dingguo Biotechnology Company (China). Fluorescamine was obtained from Fluka (Switzerland). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), gel filtration were obtained from Sigma, USA. DNA particles were supplied by Tianjin Medical University (China). All the chemicals were of analytical grade and no further purification was required.

1.2 Synthesis and characterization of lysine modified chitosan

1.2.1 Synthesis of lysine modified chitosan

Typically, 0.11 g of chitosan powder was dissolved in 10 mL of deionized water, and 3 $mol·L^{-1}$ HCl was added with the molar ratio of HCl to the amine of chitosan to be 1:10. Then lysine, EDC, and DMF were added to the solution under stirring. Polymerization was performed at constant temperature for some time. Finally, the mixture was centrifuged, and the precipitate was washed with ethanol thrice. After being purified by dialyzing in water, the solution was lyophilized, to give a light white powder.

To acquire the respective experimental conditions, such as, pH, temperature, stirring rate, ratio of oil phase and water phase (O/W), the reaction time and so on, we designed correlation experiments (Table 1).

Table 1 The correlation experiments

	m(chitosan)/g	*m*(lysine)/g	*T*/℃	Molar ratio of O/W	*t*/h	Stirring rate ($r·min^{-1}$)
(1)	0.124	0.215	0	1:1	2	1000
(2)	0.123	0.219	20	1:1	2	1000
(3)	0.122	0.218	40	1:1	2	1000
(4)	0.125	0.214	20	1:1	4	1000
(5)	0.121	0.214	20	1:1	6	1000
(6)	0.126	0.216	20	0.5:1	2	1000
(7)	0.127	0.217	20	2:1	2	1000
(8)	0.125	0.215	20	1:1	2	800
(9)	0.124	0.216	20	1:1	2	1200

1.2.2 Analysis of the amounts of amino group of CTS-lys

Free primary amino groups of CTS-lys were determined by the fluorescamine assay as previously described[11−13]. An aliquot of 100 μL of each sample was diluted with 1.4 mL assay buffer (100 $mmol·L^{-1}$ boric acid-NaOH, pH=7.0−9.5), and 500 μL 0.01% fluorescamine (prepared in acetone) was rapidly added. Samples were rapidly inverted four to five times and incubated at room temperature for 10 min. Fluorescence was measured using a Hitachi F-4500 Fluorescence Spectrophotometer with λ_{ex}=392 nm, λ_{em}=480 nm, and 5−10 nm slit widths. Background fluorescence was measured using the assay buffer only.

1.3 Synthesis of CTS-lys magnetic nanoparticles (CTS-lys-MNPs)

Solution A: 3.0 g CTS-lys (MW 40000) and 0.326 g ferric in 15.0 mL H_2O were passed through a filter (220 nm) and precooled on ice. Solution B: 0.150 g ferrous in l mL H_2O was passed through a filter (220 nm) and precooled on ice. Solution B was added dropwise into solution A under rapid stirring, followed by 5 mL 20% NaOH. Later, the solution was slowly heated to 70−90 ℃ within 1 h. The solution was kept at this temperature and stirred for another 30 min. After elimination of sodium hydroxide by dialyzing (MWCO 14000) in distilled water, CTS-lys-MNPs were separated by a magnet and the pellets were prepared using lyophilization.

1.4 Cytotoxicity assay of CTS-lys-MNPs

U293 cells (4000 cells/well) were seeded in 96-well plates. The cells were incubated for 4 h with 200 μL of complete culture medium containing CTS-lys-MNPs at different concentrations. The medium in each well was replaced with 100 μL of complete fresh medium 4 h later. After 25 μL of MTT solution in PBS (5 $g·L^{-1}$) was added, the cells were incubated for another 2 h. Subsequently 100 μL of the extraction buffer (20% sodium dodecyl sulfate (SDS) in 50% DMF, pH=4.7) was added to the wells, and the cells were incubated overnight. The optical intensity was measured at 550 nm using a microplate reader (model 550, BioRad Lab, Hercules, CA). In addition, the cytotoxicity of the pure synthetic magnetites (MNPs) was evaluated. As they were being used for gene delivery, CTS-lys-MNPs would be complexed with DNA, and the complexes would generally show less toxicity than pure MNPs[14,15].

1.5 Complexes of CTS-lys-MNPs and DNA

1.5.1 Formation of the MNPs-DNA complexes

All complexes of DNA and polymer were freshly prepared before use. CTS-lys-MNP solutions were added to the DNA solutions in equal volumes, mixed by vortexing, and incubated for 30 min before use, unless otherwise stated. DNA stock so-