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Synthesis, characterization and in vitro evaluation of methotrexate conjugated fluorescent carbon nanoparticles as drug delivery system for human lung cancer targeting

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ABSTRACT

Nanotechnology based cancer therapeutics have rapidly advanced towards the solution of many limitations associated with other drug delivery agents such as nonspecific distribution within the body, low water solubility and non-biocompatibility. Carbon nanoparticles have demonstrated unique properties that are useful to combat with these issues, including their properties dependent on size, high stability in different solvents, compatible size for drug delivery and ease of surface modifications. Fluorescent carbon nanoparticles with good water solubility were obtained from a carbohydrate source by acid assisted ultrasonic treatment at 35 kHz for 4 h. This simple and economical method can be used for large scale production. Electron microscopic, spectroscopic and thermo gravimetric analysis techniques were used to characterize these carbon nanoparticles. Functionalized CNPs were further conjugated with anticancer drug-methotrexate and used as fluorescent nano-carriers. In this research work, we determined the in vitro bioactivity of CNPs-methotrexate conjugates by lactate dehydrogenase assay, cell adhesion assay and sulforhodamine B assay in human lung carcinoma cell line (H157). The CNPs showed promising biocompatibility and CNPs-MTX conjugates demonstrated potent cytotoxic effects and high anticancer activities in human lung cancer cell line.

1. Introduction

Nanomaterials have gained increasing interest due to their useful applications in various fields of biology and medicine. With respect to their unique physical and chemical properties, metallic nanoparticles in particular played an important role in imaging and therapeutic applications [1]. Conventionally used chemotherapy agents are not distributed specifically. Targeting of the cells and tissues at molecular level has become the advanced technique to address the issues of target specificity [2]. Cytotoxicity of all types of chemotherapeutic agents may be compromised due to the development of resistance in cancer cells [3].

Nanoparticles have gained great importance because they can be fabricated in such a way to get the desirable properties [4–6]. Carbon nanomaterials can easily penetrate blood brain barrier without harming cells or undesirable effects. Carbon nanoparticles are better compared to traditional quantum dots because they have good water solubility, strong resistance to chemicals, very low toxicity and good biocompatibility [7,8]. Carbon nanoparticles have found many applications in the field of cell imaging and drug delivery due to their special features [9].

Recently, carbon nanoparticles have been prepared by different methods but the separation and purification steps were lengthy and costly. One-step ultrasonic chemical method was used for synthesizing highly fluorescent carbon nanoparticles (CNPs) from a carbohydrate directly [8]. These carbon nanoparticles are fairly soluble and chemical modifications can be achieved to attach a number of different agents [10].

Present study was focused on synthesis of CNPs from glucose and sucrose by ultrasonic method. Carbon nanoparticles were further functionalized with ethylene diamine and attached with anti cancer drug-methotrexate [11–13]. CNPs and CNPs-MTX conjugates were characterized by different microscopic and spectroscopic techniques. CNPs were used as fluorescent nano-carriers for anti-cancer drug (methotrexate). We evaluated the in vitro biocompatibility and bioactivity of CNPs and CNPs-methotrexate conjugates in human lung carcinoma cells. In vitro bioactivity and biocompatibility assessments of CNPs-MTX conjugates and CNPs, respectively, were evaluated by biological endpoints (LDH assay, cell adhesion assay

Abbreviations: CNPs, carbon nanoparticles; CNPs–MTX, carbon nanoparticles–methotrexate conjugates; EDC, 1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide.HCl; EDS, Energy dispersive X-ray spectroscopy; EN, ethylene diamine; FBS, fetal bovine serum; HBSS, Hank's balanced salt solution; LDH, lactate dehydrogenase; MTX, methotrexate; MWCNT, multi walled carbon nanotubes; PL, photoluminescence; RPMI-1640, Roswell Park Memorial medium; SWCNT, single walled carbon nanotubes; TCA, trichloroacetic acid; TGA, thermogravimetric analysis; Tris, tris(hydroxymethyl)aminomethane.

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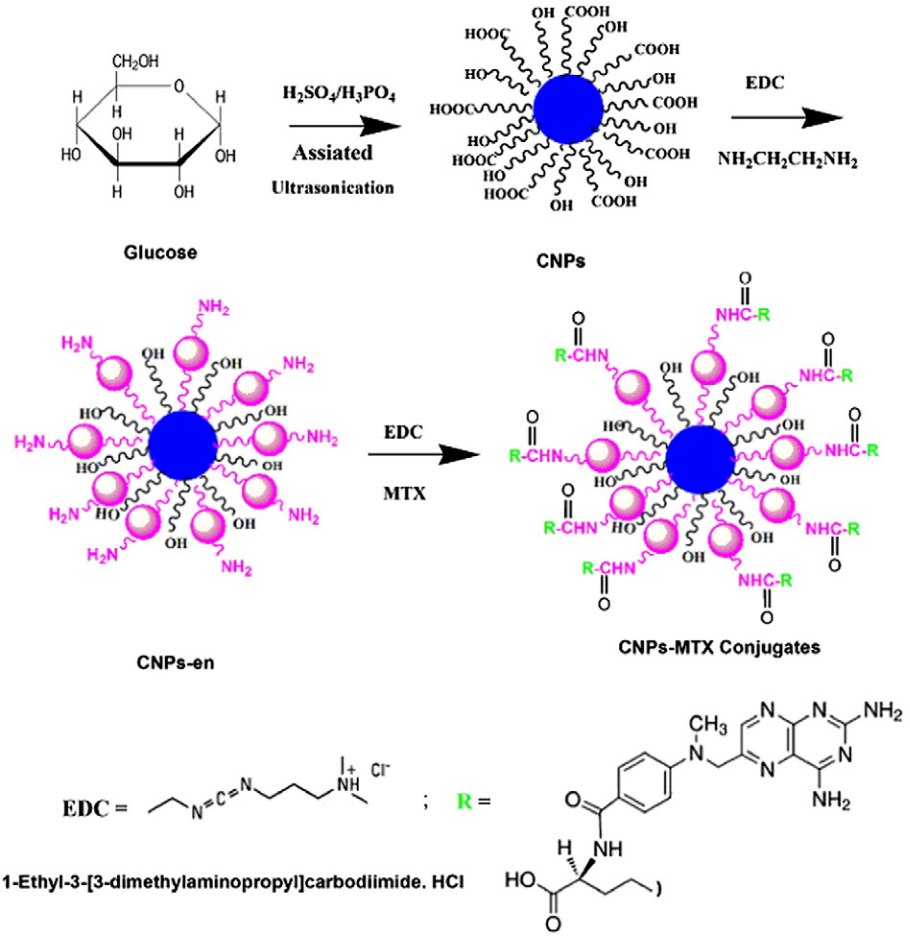

Fig. 1. Schematic diagram for synthesis, functionalization and attachment of anticancer drug-methotrexate with carbon nanoparticles derived from glucose and sucrose in acidic conditions.

and sulforhodamine B assay) in human lung carcinoma cell line-H157 [14–17]. Finally, morphological changes in H157 cells were observed under optical inverted microscope in phase contrast microscopy mode [18].

2. Materials and methods

2.1. Materials

Glucose (99%, cat. no. G8270), Sucrose (>99%, cat. no. S0389), Orthophosphoric acid (85%, cat. no. 438081), Sulphuric acid (99.9%, cat. no. 339741), methotrexate hydrochloride (cat. no. M 8407), EDC cross linker (cat. no. 7750), Hank's balanced salt solution (cat. no. 55021C), Trichloroacetic acid-TCA (cat. no. T0699) and Ethylene diamine (99%, cat. no. E26266) were purchased from Sigma Aldrich. LDH cytotoxicity assay kit (cat. no. 88953) was purchased from Thermo Scientific; and Phosphate buffered saline (cat. no. 70011-069), Fetal bovine serum (cat. no. 10437-028), RPMI-1640 medium (cat. no. 11875-093) were purchased from Invitrogen. Lung carcinoma cell lines were maintained in RPMI-1640 medium at 37 °C and 5% CO_2 incubators.

The CNPs were synthesized by using ultrasonic bath (UC-D10, BMS) and purified by centrifugation. carbon nanoparticles were characterized

Fig. 2. Typical photographs of (a) as synthesized carbon nanoparticles (b) CNPs dispersion in water under sunlight (c) CNPs dispersions under UV lamp at 365 nm (d) CNPs-MTX conjugates under UV lamp at 365 nm.