Journal of Photochemistry & Photobiology, B: Biology 182 (2018) 92–99

Contents lists available at ScienceDirect

Journal of Photochemistry & Photobiology, B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol

Improved chemo-photothermal therapy of hepatocellular carcinoma using chitosan-coated gold nanoparticles

Dina S. Salem[a], Mahmoud A. Sliem[a,*], Mohamed El-Sesy[b], Samia A. Shouman[b], Yehia Badr[a]

[a] National Institute of Laser Enhanced Science, Cairo University, Giza, Egypt
[b] National Cancer Institute (NCI), Cairo, Egypt

ARTICLE INFO

Keywords:
Hepatocellular carcinoma
Nanocarrier
Cytotoxicity
Chemo-photothermal therapy
5-Fluorouracil

ABSTRACT

A green method was used for producing gold nanoparticles (Au NPs) using chitosan as a natural cationic, biodegradable and biocompatible polymer. In this method, chitosan acts as a reducing and stabilizing agent for the synthesis of Au NPs. Different concentrations of chitosan solutions (0.01%, 0.05%, 0.1%, 0.2%, 0.5% and 1%) were applied. In an attempt to mitigate the side effects of anti-cancer drug, 5-fluorouracil (5-FU), through reducing drug doses in photothermal therapy, the formed positively-charged chitosan-wrapped Au NPs were used as a drug delivery system for negatively charged 5-FU to hepatocellular carcinoma cells (HepG2). Au NPs as well as 5-FU@Au nanocomposites were characterized with UV-VIS spectroscopy, particle size, zeta potential, Fourier transform infrared spectroscopy (FTIR), transmission electron microscopy (TEM) and High-Performance Liquid Chromatography (HPLC). The chitosan concentration was shown to be an important parameter for optimizing the dispersion of Au NPs and 5-FU@Au nanocomposites over long time. This stability offers the 5-FU@Au nanocomposites as good candidate for cancer treatment with reduced drug doses in photothermal therapy. A 72% loading-efficiency of 5-FU was obtained. Cytotoxic assay was carried out on HepG2 cell line and it reveals the effectiveness of 5-FU@Au nanocomposites in the presence and absence of laser irradiation compared with the free 5-FU. The cytotoxicity effect of free 5-FU and 5-FU@AuNPs nanocomposites was studied, and it was found that the concentration of 5-FU@Au nanocomposites required to attain 50% of inhibition growth rate was lower than that of free 5-FU in absence of laser radiation and was much lower in presence of laser radiation.

1. Introduction

Hepatocelluar carcinoma (HCC) is one of the most known fatal malignancies. Globally, HCC is the fifth common cancer and it is the second causing death with 745,500 per year due to its poor prognosis [1]. Not only a small percentage (around 25%) of patients is eligible for curative therapy, such as liver transplantation, resection, or percutaneous ablation, but also it is so expensive to patients in developing countries where its prevalence increases [2]. Chronic infection with hepatitis B virus (HBV) and hepatitis C virus (HCV), fatty liver disease, exposure to dietary aflatoxin, alcohol-induced cirrhosis, smoking, obesity, diabetes, and iron overload have been frequently reported to precede the development of HCC [3]. Currently, there is no effective treatment for advanced stages of HCC due to its aggressive nature and poor survival rate. Most chemotherapeutic drugs, *e.g.*, 5-fluorouracil [4], cisplatin [5], and doxorubicin [6] have been intravenously administrated which are always associated with sorely side effects.

Gold nanoparticles (Au NPs) have been extensively used in therapeutics and diagnosis [7,8]. Their small size, thereby the large surface-area-to-volume ratio leads to interesting physiochemical and optoelectronic properties. The historically traditional methods for the synthesis of Au NPs were Turkevitch process, in case of aqueous media, and the two-phase Brust–Schiffrin method in case of organic solvents [9,10]. More recently, a plethora of synthesis techniques was reported such as photochemical reaction [11], radiolysis [12], and sonochemical technique [13]. These methods generally used toxic chemicals which were unfavorable in biological applications. Biocompatible Au NPs could be prepared by means of natural-cationic, non-toxic, biodegradable, and biocompatible polymer, in particular the chitosan.

Chitosan has electronegative and polyelectrolyte properties. Such a dual nature renders it suitable as a reducing agent and electrostatic stabilizer [14]. Chitosan could stabilize nanoparticles through steric hindrance elevated through amino groups in its polycationic structure. Boundless amino groups of chitosan allow for ionic cross-linking to multivalent elements enabling them to be a good candidate for drug/DNA delivery [15,16]. The distinct characteristics of chitosan-coated

* Corresponding author.
E-mail address: mahmoud.ashour@niles.edu.eg (M.A. Sliem).

https://doi.org/10.1016/j.jphotobiol.2018.03.024
Received 27 January 2018; Received in revised form 29 March 2018; Accepted 31 March 2018
Available online 04 April 2018
1011-1344/

noble metallic nanoparticles such as gold and silver nanoparticles stimulated their high use in a wide span of fields [17].

Among the chemotherapeutic agents used for liver cancer treatment is 5- Fluorouracil (5-FU) which is antimetabolite drug inhibiting the normal metabolic pathway for DNA and RNA processing [18]. The antineoplastic 5-FU has been extensively used in clinical chemotherapy for cancer treatment of solid tumors such as liver, breast, ovary, colorectal, stomach, lung and pancreas [19]. The main Limitations of 5-FU are the short half-life *in vivo* [20] and the incomplete oral absorption due to its degradation by dihydropyrimidine dehydrogenase (DPD) [4,21]. In addition, the nonselective action of 5-FU against normal cells constitute a critical limitation of its application. Therefore, high effective dose is required for treatment which can increase the harmful side effects.

In an attempt to overcome the present limitations of 5-FU, chitosan coated gold nanocarrier was applied. Beside the role of Au NPs as nanocarriers, they absorb and scatter visible laser light efficiently causing local hyperthermia resulting in killing of cancer cells as photothermal agents [22,23].

Au NPs are, on the other hand, unique for their enhanced permeation retention effect, as they highly accumulate in cancer cells rather than the health ones which make Au NPs ideal as drug delivery cargo [24]. Consequently, this renders the photothermal therapy (PTT) highly selective to cancer cells in the presence of Au NPs as photothermal agents.

In the current manuscript, we assessed the effectiveness of drug-assisted PTT as a combined cancer treatment method. The efficacy of chitosan-coated 5-FU@Au nanocomposite as an *in vitro* drug cargo in presence and absence of light was examined, in comparison to free drug 5-FU.

2. Material and Methods

2.1. Chemicals

Hydrogen tetrachloroaurate trihydrate ($HAuCl_4{\cdot}3H_2O$, 98%, molecular weight: 393.83), Low-molecular-weight chitosan with degree of deacetylation of 75%, Tripolyphosphate ($Na_5P_3O_{10}$, TPP, molecular weight: 367.86) and anticancer drug (5-FU) were purchased from Sigma–Aldrich, USA with high grade of purification. Acetic acid was obtained from Merck and diluted before used to be 1% aqueous solution.

2.2. Preparation of Chitosan Wrapped Gold Nanoparticles

A stock solution of chitosan (1%) was prepared by dissolving 1 g of chitosan powder in 1% acetic acid. The clear solution was obtained by keeping it stirring overnight at room temperature. A monodispersive Au NPs was synthesized by reduction of gold solution (125 µL, 0.01 M) in different concentrations of chitosan solution (0.01, 0.05, 0.1, 0.2, 0.5 and 1%) at 100 °C. Upon reduction, the color changed gradually from colorless to pale red and finally to ruby-red color, confirming the formation of Au NPs. The as-prepared Au NPs were characterized with UV-VIS spectroscopy, particle size, poly dispersity index (PDI), zeta potential, Fourier transform infrared (FTIR), and Transmission electron microscopy (TEM).

2.3. Preparation of Drug-loaded Gold Nanoparticles

The as-prepared Au NPs, using 0.2% chitosan, was mixed with 5-FU (0.001 M) dissolved in double distilled water with a ratio of 1:1 (v/v). The solution was stirred for 6 h at room temperature to promote the loading of 5-FU on the surface of chitosan-coated Au NPs. The obtained nanocomposites (5-FU@Au) were characterized with UV-VIS spectroscopy to inspect the variations in shape, size and size distribution. Further, particle size and zeta potential analysis were measured to detect the stability of 5-FU@Au nanocomposites through their surface charges. In addition, Fourier transform infrared (FTIR) was utilized to collect information about the loading process. Transmission electron microscopy (TEM) was used to elucidate the shape and size of these nanoparticles as well as High-Performance Liquid Chromatography (HPLC) to determine loading efficiency.

2.4. UV–VIS Spectral Analysis

The changes in the surface plasmon resonance (SPR) of Au NPs and 5-FU@Au nanocomposites were recorded using Cary-Varian 50 Bio UV–visible spectrophotometer. Diluted solutions of samples were placed in 1 cm UV-quartz cuvette and the absorption was recorded within appropriate scan range.

2.5. Particle Size and Zeta Potential Measurements

Hydrodynamic diameter, Polydispersity index (PDI), and surface charge of Au NPs prepared from different concentrations of LMW chitosan were analyzed by dynamic light scattering (DLS) using ZS-ZEN (Malvern Instruments Co., UK). The samples were measured at a scattering angle of 173° at room temperature. The measurement of each sample was repeated three times.

2.6. Transmission Electron Microscopy (TEM)

TEM images of Au NPs and 5-FU@Au nanocomposites were captured on JEM-1400 transmission electron microscope (TEM; JEOL Ltd., Tokyo, Japan) coupled with CCD camera model AMT operating at 80 KV. First, samples pipetting up and down to be resuspended. Then, 2–5 µL drops of samples were mounted on carbon-coated 400-mesh copper grids. The specimens were left to dry for 2 min. Filter paper was used to remove excess solution and facilitate the settle down of particles on the grids.

2.7. Fourier-Transform Infrared Spectrophotometry (FTIR)

Infrared spectra of 5-FU and 5-FU@Au nanocomposites were measured on FT/IR-4100 type A instrument (JASCO, Tokyo, Japan) equipped with triglycine sulfate detector (TGS) detector. The powdered samples were mixed with KBr pellets and scanned in transmission mode in the spectral range of (4000–500 cm^{-1}) at resolution of 4 cm^{-1} and 2 mm/s scanning speed.

2.8. Chromatography

Quantification of 5-FU was performed by YL9100 HPLC system equipped with YL9120 UV/Vis detector, YL9101 vacuum degasser model and YL9110 quaternary pump. The HPLC assay was carried out on stationary phase, Eclipse Plus C18 column (150 × 4.6 mm, i.d 5 µm pore size, Agilent ZORBAX, USA). The column temperature was maintained at room temperature. Isocratic mobile phase mixture consisted of two eluents, methanol (30%) and acidified water (100 mL H_2O + 150 µL Phosphoric acid) (70%) was used. The flow rate was 0.9 mL/min with 20 µL sampling injection. Detection of 5-FU was performed at 266 nm wavelength. The entire run time was 4 min per sample.

2.9. In vitro Cytotoxicity Assay

Cytotoxicity of free 5-FU, Au NPs, and 5-FU@Au nanocomposites was evaluated in absence and presence of laser irradiation on human hepatocellular carcinoma cells (HepG2) cell line at the Pharmacology Unit, National Cancer Institute (NCI), Cairo University, Egypt. HepG2 cells were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. Cells were seeded into 96-well plate at a density of 5×10^3 per well and were incubated