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Colloidal HSA – Graphene oxide nanosheets for sustained release of oxaliplatin: Preparation, release mechanism, cytotoxicity and electrochemical approaches

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ABSTRACT

A novel sustained release carrier of graphene oxide nanosheets functionalized by human serum albumin nanoparticles (FGO-NSs) was synthesized and characterized by field emission scanning electron microscopy (FESEM), zeta sizer analysis, absorption spectroscopy (UV–vis) and Fourier transform infrared spectroscopy (FTIR) studies. The cell viability of GO-NSs, HSA-NPs and FGO-NSs has been tested against human foreskin fibroblast normal cell line, HFFF2 by MTT assay technique. Loading efficiency and release properties of oxaliplatin (OX), as an effective chemotherapeutic agent in the treatment of cancers, from HSA-NPs and FGO-NSs were studied. The results showed high drug loading (DL), drug entrapment efficiency (DEE) and sustained release of OX from FGO-NSs (DL = 61 ± 4% and DEE = 1.2 ± 0.2%) in comparison with HSA-NPs (DL = 51 ± 3% and DEE = 0.9 ± 0.04%) due to the large surface area of GO sheets. The diffusion coefficient was evaluated by cyclic voltammetry (CV) and confirmed the obtained results of UV–vis spectroscopy. Finally, release mechanism studies indicated that the release of OX from HSA-NPs is controlled by Fickian diffusion while the release mechanism of FGO-NSs/OX is occurred through non-Fickian diffusion. The results showed that the FGO-NSs could be suitable for reducing cytotoxicity of GO-NSs and enhancement of drug loading and sustained drug release in comparison with HSA-NPs in cancer therapy.

1. Introduction

The development of nanotechnology and molecular biology has provided the improvement of nanomaterials with specific properties which are now able to overcome the weaknesses of traditional disease diagnostic and therapeutic procedures [1]. In recent years, more attention has been devoted to designing and the development of new methods for realizing sustained release of diverse drugs. Since each drug has a plasma level above which is toxic and below which is ineffective and in conventional drug delivery, the drug concentration in the blood rises quickly and then declines, the main aim of an ideal drug delivery system (DDS) is to maintain the drug within a desired therapeutic range after a single dose, and/or target the drug to a specific region while simultaneously lowering the systemic levels of the drug [2–4].

Graphene–based materials such as graphene oxide (GO) have considerable potential for several biological applications including the development of new drug release system. GOs are an abundance of functional groups such as hydroxyl, epoxy, and carboxyl on its basal surface and edges that can be also used to immobilize or load various biomolecules for biomedical applications [1,5,6]. On the other side, biopolymers have frequently been used as raw materials for designing drug delivery formulations owing to their excellent properties, such as non-toxicity, biocompatibility, biodegradability and environmental sensitivity, etc [7]. Protein therapeutics possess advantages over small-molecule approaches including high target specificity and low off target effects with normal biological processes [8]. Human serum albumin (HSA) is one of the most abundant blood proteins. It serves as a transport protein for several endogenous and exogenous ligands as well as various drug molecules [9,10]. HSA nanoparticles have long been the center of attention in the pharmaceutical industry due to their ability to bind to various drug molecules, high storage stability and *in vivo* application, non–toxicity and antigenicity, biodegradability, reproducibility, scale–up of the production process and a better control over release properties. In addition, significant amounts of drugs can be incorporated into the particle matrix because of the large number of

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drug binding sites on the albumin molecule [4]. Therefore, the combination of HSA-NPs and GO-NSs could be useful for reducing the cytotoxicity of GO-NSs and the enhancement of drug loading and sustained drug release in cancer therapy.

In this study, we have prepared graphene oxide nanosheets (GO-NSs) using Hummers method and HSA nanoparticles (HSA-NPs) by modified desolvation method. In addition, we have prepared FGO-NSs as a novel anticancer drug carrier system to achieve a prolonged therapeutic effect. The characterization of nanostructures is performed by FTIR, UV–vis and FESEM studies. The cell viability of HSA-NPs and FGO-NSs is evaluated on human foreskin fibroblast normal cell line by conducting MTT assays. The *in vitro* drug release is carried out and the drug loading efficiency and entrapment efficiency in HSA-NPs and FGO-NSs is determined. In addition, the mechanism of drug release and the best fit kinetic model are investigated.

2. Experimental

2.1. Materials

Human serum albumin (HSA), Graphite (200mesh), phosphate buffer saline tablet (PBS) and glutaraldehyde solution were obtained from Sigma Aldrich Company (USA). The following chemicals were reagent grade and used as purchased from Merck Chemicals CO (Germany); Potassium permanganate, sulfuric acid, hydrochloridric acid and hydrogen peroxide. Oxaliplatin (OX) was purchased from Pfizer Pharmaceuticals Group. The human foreskin fibroblast normal cell line, HFFF2, used in cytotoxicity studies were obtained from the Cell Bank of the Pasteur Institute in Tehran (Iran). All other reagents and solvents were of analytical grade and double distilled water was used for all experiments.

2.2. Methods

The Fourier Transform Infrared (FTIR) spectra were recorded using a Jasco-460 plus FTIR spectrometer in KBr disk in the range of 4000-400 cm^{-1}. UV–vis spectra were performed by a UV–vis spectrometer (Perkin Elmer Lambda 25, USA). The zeta-potential measurements were performed by a Malvern Instruments Zeta Sizer 3000 HSa using a standard rectangular quartz cell. Field Emission Scanning Electron Microscopy (FESEM) measurements were obtained by Mira 3-XMU in order to acquire topographical and elemental information at specific magnifications with a virtually unlimited depth of field. Parstate 2273 Potentiostate was used for cyclic voltammetric (CV) measurements. The three-electrode system was used for electrochemical studies, this system contains glassy carbon (GC) as the working electrode, a Pt wire and saturated calomel electrode as the counter and reference electrodes, respectively. For reproducible experimental results numerous voltammograms were recorded until the achievement of a steady state baseline. pH measurements were performed by a Metrohm pH-meter, model 826 pH mobile.

2.3. Preparation of human serum albumin nanoparticles

Human serum albumin nanoparticles)HSA-NPs(were prepared by desolvation technique with some modification [4]. 50 mg of HSA was dissolved in 2 mL of deionized water and stirred at 500 rpm using a magnetic stirrer at room temperature for 10 min. Ethanol (desolvating agent) was gradually added to HSA solution with a rate of 0.5 mL/min. Then, 0.012 mL of glutaraldehyde 8% (one of the cross linked agents) was added to the solution and stirred continuously (500 rpm) at room temperature for 20 hours. The prepared nanoparticles were purified by 5 cycles of centrifugation at 14,000 rpm for 10 min. Finally, the nanoparticles were resuspended in 2 mL of deionized water.

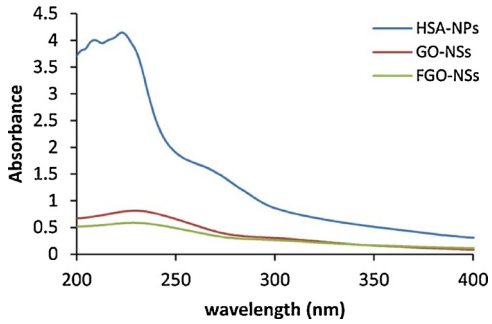

Fig. 1. UV–vis spectra of HSA-NPs, GO-NSs and FGO-NSs.

2.4. Preparation of graphene oxide nanosheets

Graphene oxide nanosheets (GO–NSs) were synthesized from natural graphite by modified Hummer's method [7]. Graphite powder (500 mg) was first added to 24 mL of concentrated sulfuric acid under 0 °C temperature (in an ice bath) and was stirred for 15 min, and then 3 *g* of $KMnO_4$ was added to the mixture. The reactor was then transferred to a 40 °C bath and was completely stirred for 24 h. 100 mL water was then added to the system and the mixture was stirred in ambient temperature. 3 ml H_2O_2 was added under slow stirring until the color of the solution changed to yellow form dark brown. Then the mixture was centrifuged and washed three times with 10% HCl solution and then three times with water. The procedure was followed by sonication for 30 min to produce a stable aqueous dispersion of GO–NSs. At the end of this process, the mixture was centrifuged for 15 min at 4500 rpm in order to remove un-exfoliated graphite oxide particles. The solution was then washed with water until the pH reached 5.0.

2.5. Preparation of functionalized graphene oxide nanosheets

Functionalized Graphene Oxide Nanosheets (FGO-NSs) were prepared with a slight modification in the protocol of HSA-NPs preparation [11–13]. After the disolvation step, 50 mg of graphene oxide (GO) was added to the solution (1:1 HSA/GO). Then, 0.012 mL of glutaraldehyde 8% was added into the solution and the reaction continued for 20 hours with continuous stirring (500 rpm) at room temperature. The solution was centrifuged 5 cycles at 14,000 rpm for 10 min. The FGO-NSs were sonicated after each cycle for 10 min. Finally, the FGO-NSs were re-suspended in 2 mL of deionized water.

2.6. Cell viability measurements

Cell proliferation was evaluated by using a system based on the tetrazolium compound [3-(4, 5-dimethylthia-zol-2-yl)-2, 5-diphenyltetrazolium bromide, MTT] which was reduced by living cells to yield a soluble formazan product which can be assayed colorimetrically [14]. The MTT assay is dependent on the cleavage and the conversion of the soluble yellowish MTT to the insoluble purple formazan by active mitochondrial dehydrogenase of living cells. The human foreskin fibroblast normal cell line, HFFF2, was maintained in a RPMI medium supplemented with 10% heat-inactivated fetal calf serum and 2 mM L-glutamine, streptomycin and penicillin (5 μg mL^{-1}), at 310 K under a 5% CO_2/95% air atmosphere. Harvested cells were seeded into 96-well plate with the concentration of the sterilized nanoparticles (6.25 mg mL^{-1}) and incubated for 24 h. At the end of the four hour incubation period, 25 μL of MTT solution (5 mg mL^{-1} in PBS) was added to each well containing fresh culture media. The insoluble produced formation was then dissolved in a solution containing 10% SDS and 50% DMF (under dark condition for 2 h at 310 K) and optical density (OD) was read against the blank reagent which was measured at 570 nm by Microplate reader. Absorbance was read as a function of