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Preparation, characterization and cytotoxicity of schizophyllan/silver nanoparticle composite

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ABSTRACT

Silver nanoparticles (Ag-NPs) have been successfully prepared with a simple and "green" chemical reduction method. Triple helical schizophyllan (SPG) was used for the first time as reducing and stabilizing agents. The effect of temperature, silver nitrate/schizophyllan concentrations, pH of the reactions medium and the reaction time were investigated. The obtained schizophyllan/Ag-NP was characterized by UV–vis spectroscopy, TEM, DLS, X-ray diffraction, TGA, and ATR-FTIR. The results revealed that, Ag-NPs attached to SPG through a strong non-covalent interaction, leading to good dispersion of Ag-NPs with a diameter of 6 nm within the biopolymer matrix. By increasing the pH of the reaction medium, the triple helical structure of SPG was partially broken. The SPG/AgNP nanocomposite was non-toxic for mouse fibroblast line (NIH-3T3) and human keratinocyte cell line (HaCaT).

1. Introduction

Metal nanoparticles and their polymer composites represent an important field for fundamental studies offering wide range of engineering and biomedical applications. Due to the large surface area of common nanoparticles (NPs), controlled dispersion of NPs in the matrix remains one of the main difficulties. When the NPs were used alone, they showed strong aggregation resulting in a significant reduction of their surface activity (Zhu et al., 2002). To reduce the NP aggregation, preparation of nanoparticle compounds, in which individual NPs are distributed within the suitable polymer matrix, is one of the most effective approaches (Choy, Park, & Yoon, 1998; Miao et al., 2006; Mogyorósi, Dékány, & Fendler, 2003).

Natural polymers such as sodium hyaluronante (Abdel-Mohsen, Hrdina, et al., 2012; Abdel-Mohsen et al., 2013), chitosan and/or chitosan derivatives (Abdel-Mohsen, Abdel-Rahman, et al., 2012; Abdel-Mohsen, Aly, Hrdina, & El-Aref, 2012; Fouda, El-Aassar, & Al-Deyab, 2013), and cellulose are all important macromolecular materials increasingly used in biomedical applications due to their inherent biodegradability with degradation products which is non-toxic for the cells.

There are several methods for preparation of metal and metal oxide nanoparticles, such as photo-chemical reduction (Darroudi, Ahmad, Shameli, Abdullah, & Ibrahim, 2009), laser libations (Darroudi et al., 2011), chemical reduction with different polymers/biopolymers (Aihara, Torigoe, & Esumi, 1998; El-Rafie et al., 2011; Lin, Teng, & Yang, 2003), reduction of chemicals in soft and stiff matrices (Darroudi, Ahmad, Abdullah, Ibrahim, & Shameli, 2010; Hebeish, El-Rafie, Abdel-Mohdy, Abdel-Halim, & Emam, 2010; Zhuang, Cheng, Kang, & Xu, 2010) reduction of chemicals in staple matrices (e.g., porous silicate) (Dag, Samarskaya, Coombs, & Ozin, 2003), and chemical deposition (Szłyk et al., 2001; Textor, Fouda, & Mahltig, 2010).

Environmentally friendly (green) way to prepare silver nanoparticles (Ag-NPs) depend on three important parameters, such as solvent medium, reducing and stabilizing or capping agent for Ag-NPs (Abdel-Mohsen, Hrdina, et al., 2012; Hebeish et al., 2011). Many methods reported to use external reducing agents for preparation of silver nanoparticles. The use of toxic or harmful reducing

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agent, such as sodium borohydride, results in tiny particles that are well-dispersed (Ji, Chen, Wai, & Fulton, 1999; Shah, Holmes, Doty, Johnston, & Korgel, 2000). Nowadays, a succession chemical reduction method is used for the synthesis of noble metal nanoparticles such as, sodium borohydride (Shameli et al., 2010; Shen et al., 2010), ammonia solution (Lim, Jiang, Yu, Camargo, & Xia, 2010), diazine (Mayer, Grebner, & Wannemacher, 2000; Underhill & Liu, 2000), dimethyl aminoborane (Torigoe, Suzuki, & Esumi, 2001), hydrogen (Henglein & Giersig, 2000), HCHO, $HCONH_2$ (Yin et al., 2002), C_2H_5 (Wang, Neoh, & Kang, 2001; Wang, Ren, Deng, Gui, & Tang, 2000), $C_5H_5O_7Na$ (Pathak et al., 2000), and ascorbic acid (Lim et al., 2010). Schizophyllan (SPG), as biopolymer, is an extracellular linear β-1,3-glucan with one β-6-1-linked glucose residue per three main chain glucose residues produced by the fungus *Schizophyllum commune*. The chemical structure was shown in Fig. 1D-I. Norisuye and coworkers (Kashiwagi, Norisuye, & Fujita, 1981; Norisuye, 1985; Norisuye, Yanaki, & Fujita, 1980; Sato, Norisuye, & Fujita, 1981; Yanaki, Norisuye, & Fujita, 1980) studied the properties of schizophyllan biopolymer in details and they found that, SPG has triple helical structure shape, and the helical structure of SPG was stabilized by inter and/or intra hydrogen bonds among with the glucan chains in SPG, which are interconnected inside the helix, whereas one glucan residue per each repeating unit was directed outward the helix core into water as a solvent. The triple helical structure of SPG was found to be interacted in water even at 120 °C (Sato, Norisuye, & Fujita, 1983) and can be destroyed only in mixture of 85% DMSO/H_2O (Sato et al., 1983). Based on this finding; no structural transition above 10 °C in water was observed. In the present preparation method (SPG) was used, as the first time, for the preparation of silver nanoparticles (Ag-NPs), serving as reducing and capping agent for the formed Ag-NPs. Water was also utilized as the environmentally benign solvent throughout the preparation of Ag-NPs. The effect of reaction condition, including the pH value, temperature, and concentrations of both $AgNO_3$, SPG, and time on the size and morphology of AgNPs are discussed. Furthermore, the SPG/Ag-NPs nanocomposite structure was investigated employing TEM, X-ray diffraction, XRD, UV–vis spectra. The cytotoxicity of SPG/Ag-NPs nanocomposite was evaluated against NIH-3T3 and HaCaT cells.

2. Experimental

2.1. Materials

Schizophyllan extracted from *S. commune* weight (1.6 MDa, by SEC-MAALS) was purchased from CPN Ltd., Czech Republic. Sodium hydroxide and silver nitrate were obtained from Sigma–Aldrich, Germany. Deionized water was used for all experiments. All other solvents were used without further purification.

2.2. Methods

2.2.1. Preparation of silver nanoparticles by SPG

Desired amount of SPG was dissolved in 100 ml deionized water using heated magnetic stirrer and, after being completely dissolved, it was heated at reaction temperature ranging from 40 to 90 °C. The $AgNO_3$ solution with contraction ranging from 0.1 to 3 mg/ml was added drop-wise to the SPG solution (0.1–1%) under continuous stirring for 20–240 min. After adding the $AgNO_3$ solution, the SPG solution acquires light yellow color indicating the reduction of silver nitrate to Ag-NPs, after completing the reaction; the solution was centrifuged at 10,000 rpm for 15 min to remove the precipitate. The suspension of SPG/Ag-NP composite was then filtered through a 0.22 μm pore size filter paper followed by freeze-drying. To obtain the nanocomposite for the characterization, the SPG/Ag-NP was dissolved in deionized water (Millipore). Reaction conditions affecting the shape and size of Ag-NPs were studied and are discussed in the following section.

2.3. Characterization of schizophyllan–silver (SPG/Ag-NPs) nanoparticle composite

2.3.1. UV–visible (UV–vis) spectroscopy

UV–vis spectra measurement was carried out on UV-160A, Shimadzu, Japan) using quartz cuvettes with an optical path of 1 cm. The concentration of the measured solutions was kept at 0.4 mg/ml.

2.3.2. Transmission electron microscopy (TEM)

TEM images were observed on a JEOL JEM-2010 (HT) electron microscope, using an accelerating voltage of 150 kV. The sample was dissolved in deionized water (Millipore) with concentration of 0.4 mg/ml, and a drop was placed on Cu grids pre-coated with carbon films.

2.3.3. Dynamic light scattering (DLS)

Dynamic light scattering measurement was performed by a Malvern Zetasizer Nano ZS. The sample was filtered using a 0.45 μm nylon syringe filter directly into a polyacrylic cell.

2.3.4. X-ray diffraction (XRD)

XRD data were collected on a D8 Advance diffractometer (Bruker AXS, Germany) with Bragg–Brentano θ–θ goniometer (radius 217.5 mm) equipped with a secondary beam curved graphite mono-chromator and Na(Tl) I scintillation detector. The generator was operated at 40 kV and 30 mA. The scan was performed at room temperature from 10° to 80° (2θ) in 0.02° step with a counting time of 8 s per step.

2.3.5. Thermal analysis

The kinetics of thermal decomposition was investigated using different heating rate thermo-gravimetric (TG, Netzsch 209F3 instrument, Al_2O_3 crucible) and under heating rates of 5 °C min^{-1} (with data collecting rate of 40 points/K).

2.3.6. Attenuated total reflectance Fourier transforms infrared spectroscopy (ATR-FTIR)

Attenuated total reflectance Fourier transforms infrared spectroscopy (ATR-FTIR) spectroscopy was performed by using a Nicolet Impact 400 D FTIR spectrophotometer (Nicolet CZ, Prague, Czech Republic) equipped with a ZnSe crystal for the ATR-FTIR spectroscopy. Transmittance was measured as a function of the wave number between 4000 cm^{-1} and 600 cm^{-1} with the resolution of 8 cm^{-1} and the number of scans equal to 512.

2.3.7. Cell viability assay

As described by Vistejnova 2009 "3000 (3T3) and 4000 (HaCaT) cells/were seeded to 96-well test plates". "The cells were cultured for 24 h before treated with the cell solution". The tested solution was added to each well so that the final concentration of the tested solution in the well was 100–1000 μg/ml by diluent medium. Cytotoxicity was measured after 0, 24, 48, 72 h after the treatment with 3-(4.5-dimethylthiazol-2-yl)-2.5-diphenyl tetrazolium bromide assay. MTT is reduced by viable cells to a colored formazan salt, which the later released from the cells and determined spectrophotometrically. MTT assay was described in previously work (Vistejnova et al., 2009).

Also described by Vistejnova et al., 2009 "MTT stock solutions were added to the cell culture medium/plates were incubated at 37 °C for 2.5 h. The supernatant was discarded and cells were lysed