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Binding analysis of carbon nanoparticles to human immunoglobulin G: Elucidation of the cytotoxicity of CNPs and perturbation of immunoglobulin conformations

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ABSTRACT

The chemical compositions, sizes and fluorescent properties of synthesized carbon nanoparticles (CNPs) were characterized. *Escherichia coli* (*E. coli*) cells were used as a model to study the cytotoxicity of CNPs, and the results of the cellular uptake of CNPs yielded excellent results: the CNPs demonstrated good biocompatibility and were non-toxic to the growth of the *E. coli* cells. Moreover, to assess the potential toxicity of CNPs to human health, the binding behavior of CNPs with human immunoglobulin G (HIgG) was examined by fluorescence quenching spectroscopy, synchronous fluorescence spectroscopy and circular dichroism spectroscopy under physiological conditions. The fluorescence quenching constants and parameters for the interaction at different temperatures had been calculated according to Scatchard. The thermodynamic parameters, such as enthalpy change (ΔH), entropy change (ΔS) and free energy change (ΔG), were calculated, and the results indicated strong static quenching and showed that van der Waals forces, hydrogen bonds and hydrophobic interactions were the predominant intermolecular forces stabilizing the CNP–HIgG complex. Synchronous fluorescence and circular dichroism spectra provided information regarding the conformational alteration of HIgG in the presence of CNPs. These findings help to characterize the interactions between CNPs and HIgG, which may clarify the potential risks and undesirable health effects of CNPs, as well as the related cellular trafficking and systemic translocation.

1. Introduction

In recent years, nanotechnology using materials and structures with nanoscale dimensions has offered great promise for delivering new technologies, and the number of scientific papers describing new synthetic pathways and applications of nanoparticles has increased [1–3]. Carbon nanoparticles (CNPs) with interesting photoluminescence properties have received considerable attention [4]. Compared to commercialized quantum dots, CNPs show similar photo-physical performance and photochemical stability, but without the burden of intrinsic toxicity or elemental scarcity, presenting great potential in bioanalytical and analytical applications, such as cellular labeling [5,6], molecular imaging [7,8] and drug delivery [9]. However, as a new type of carbon-based nanomaterials, the potential toxicity of CNPs with regard to human health warrants further and more thorough investigations.

Immunoglobulin G (IgG) is a homologous protein. It has an antibody function and acts as recognizing blocks or structures to identify a foreign body or infection and initiate an immune response [10–12]. As the most therapeutically important immunoglobulin, human IgG (HIgG) can serve as a potential drug transporter and has an important role in binding metabolites, drugs, organic compounds and relevant antigens [13]. Any alteration to the structure of HIgG can hinder the function of immunoglobulins and affect their contribution to humoral immunity [14]. Thus, studies on the binding behaviors of nanoparticles with HIgG are an important field in toxicology, and investigation of the interaction between CNPs and HIgG is necessary to understand the biological effects of CNPs on the human body. Keya Chaudhuri et al. investigated the extent of perturbation of serum albumin conformations caused by CNPs and the thermodynamic parameters between CNPs and serum albumin [15]; Mandal et al. performed a simple method for the synthesis of ultrafine CNPs and examined its interaction with bovine serum albumin (BSA) [16].

Optical techniques that are sensitive and relatively easy to use are powerful tools for monitoring the interactions between nanoparticles and proteins [17,18]. Liu et al. studied the interactions between CdTe quantum dots coated with different ligands and human serum albumin (HSA) using fluorescence spectroscopy, circular dichroism (CD) spectroscopy, UV–vis spectroscopy and dynamic light scattering [19]; Naveenraj et al. studied the interaction of synthesized gold nanoparticles with serum albumins using UV–vis spectroscopy, atomic force microscopy (AFM) and transmission electron microscopy (TEM) [20].

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Table 1
Elemental analysis of the raw canola oil soot and CNPs.

Elements	Canola oil soot	CNPs
C (%)	90.51	25.64
H (%)	1.93	1.78
O (calculated) (%)	4.57	69.21
N (%)	2.99	3.37

In this paper, because of their wide existence range and sensitive reflection of environmental change, *Escherichia coli* (*E. coli*) cells were used as a model to understand the cytotoxicity of CNPs without any further functionalization. Furthermore, the interaction of CNPs with HIgG was demonstrated using fluorescence quenching spectroscopy, synchronous fluorescence and CD spectroscopy under physiological conditions for the first time. The binding constants, numbers of binding sites and basic thermodynamic parameters under different temperatures were calculated according to Scatchard plots and the Van't Hoff equation. The alterations to the protein secondary structures induced by the addition of CNPs were also examined.

2. Experimental methods

2.1. Chemicals and reagents

Canola oil from a local market was used in this work. Raw canola oil soot was collected by placing a glass plate on top of a smoldering diesel lamp. Dialysis bags (MW cutoff = 8 kDa) were purchased from Millipore Corporation (Billerica, USA). HIgG (purity >99%) was purchased from Sigma Chemical Company and used without further purification. Tris (tris(hydroxymethyl)-amino-methane) was purchased from Tianjin Guangfu Fine Chemical Research Institute (Tianjin, China). All solutions of HIgG were prepared in pH 7.40 buffer solution,

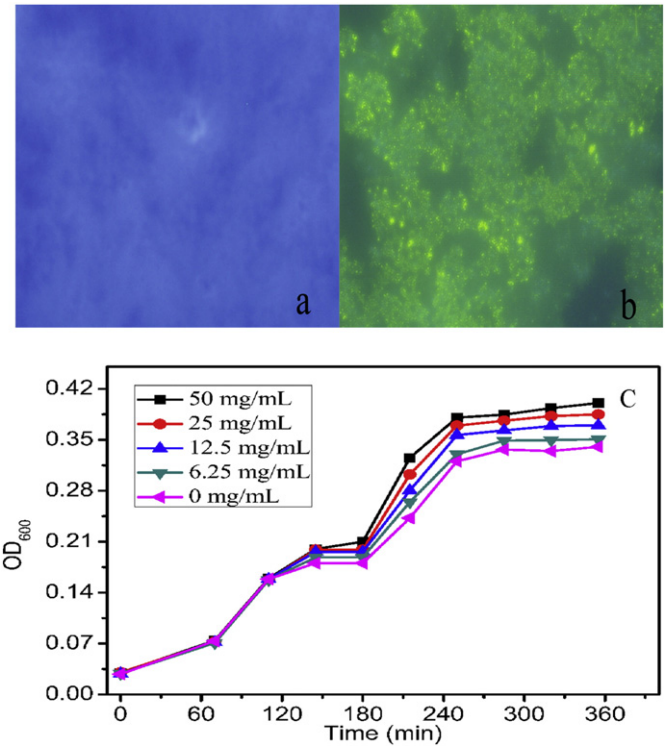

Fig. 2. Fluorescent images of *E. coli* cells (a) and *E. coli* cells incubated with CNP solution for 24 h (b), UV excitation (405 nm); (c) OD 600 values of non-treated and CNP-treated *E. coli* cells.

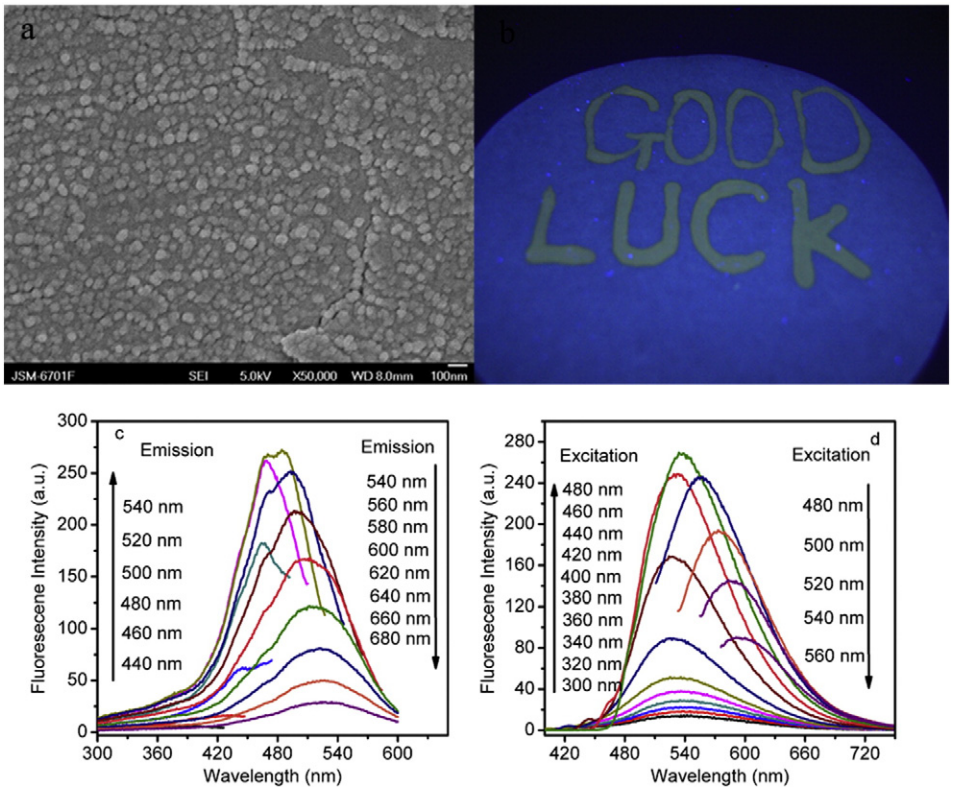

Fig. 1. SEM image of CNPs (a); fluorescent images of CNPs on commercially available paper under UV light (405 nm) (b); excitation (c) and emission (d) spectra of CNPs excited at different wavelengths.