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# Arresting cancer proliferation by controlling the surface crystallinity of carbon materials without generating reactive oxygen species

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ABSTRACT

This study demonstrated that the surface crystallinity of carbon nanostructures is an additional independent factor that should be considered for the inhibition of cancer proliferation without activating reactive oxygen species (ROS). In addition, cytotoxic evaluation of both proliferating cancer cells and fully differentiated nerve cells (i.e. non-proliferative) showed selective cytotoxicity: single-walled and highly crystalline carbon nanostructures aggressively inhibited the proliferation of glioma cancer cells, but exhibited no notable cytotoxicity effects on differentiated nerve cells. Although single-wall carbon nanotubes have been shown to elicit potent proinflammatory responses by means of trigger ROS, our results demonstrated that highly crystalline carbon structures can be utilized as a selective antiproliferative agent against brain tumor cells without increasing the ROS level and without significant cytotoxic effects to adjacent nerve cells.



## 1. Introduction

Carbon nanomaterials (CNMs) have attracted much interest over the past decade for their utilization in drug-delivery systems [1–6], which is made possible by their intrinsic nanosize and chemically stable ($sp^2$ bonding) material properties. Although some enzymes trigger CNM degradation, CNMs are considered to be very durable materials and do not produce significant toxicological chemicals in neighboring cells as well as lessening inflammation [7,8]. In addition, surface modification, such as functionalization and bioconjugation techniques, can enhance CNM solubility to ultimately elevate cellular uptake efficiency and increase gene- or drug-loading capacity to targeted cells [9–11].

Conventional toxicity evaluations of CNMs have been widely studied by other groups. For example, an in vivo study on aerosols observed the accumulation of single-walled (SWCNTs) and multi-walled carbon nanotubes (MWCNTs) in the lung, which induced acute and cytotoxic inflammatory responses that culminated in the formation of fibrosis tissues (fibrosis) [12–14]. In vitro studies of human skin fibroblasts on CNMs have also showed activation of genes associated with stress response, cellular metabolism, transport and cell-cycle regulation [15]. Furthermore, studies have examined the mechanism by which CNMs induce cytotoxicity. For example, CNMs damage the plasma membrane in macrophages [16], alter the paracellular permeability of human airway epithelial cells [17] and activate caspase (an inducer of apoptosis) by raising oxidative stress in rat lung epithelial cells [18–21].

However, most of the previous studies have focused on nanotoxicity in terms of size [22], aggregation [23,24] and chemical functionality [25,26], but have not studied surface crystallinity in detail [27]. Blood and nanoparticle interactions are critical issues [28–30] that must be addressed in the evaluation of CNM cytotoxicity, as blood serum proteins interact nearly instantaneously with nanomaterials once they are introduced into the blood vessel.

As such, it is imperative to address the intrinsic toxicity induced by the conjugation of nanoscale materials with serum proteins on targeted cells. This study demonstrated the cytotoxic effects of CNMs of varying size and crystallinity on proliferating glioma cells (i.e. brain cancer cells). The toxicology and viability of fully differentiated brain nerve cells (i.e. non-proliferating) was also evaluated. Different sized CNMs, e.g. SWCNTs, MWCNTs and two types of carbon nanofibers (CNFs) (i.e. low-crystalline CNF (LCCNF) and highly crystalline CNF (HCCNF) by heat treatment), were dispersed in serum proteins in order to evaluate the cytotoxicity on both human glioma (U373MG) and fully differentiated nerve cells (HT22).

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