Neurotoxic effect of non-functionalized carbon nanospheres

Efecto neurotóxico de las nanoesferas de carbono no funcionalizadas

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Abstract

Objective. Different types of nanoparticles, including carbon ones, were tested for a variety of biological applications. Alongside with promising results, some undesired side effects were disclosed. Most biological studies were performed on highly functionalized carbon nanospheres, so the main objective of this study was to assess cytotoxicity of unfunctionalized carbon nanospheres synthesized by sol-gel method. We hypothesize that unfunctionalized material will present different cytotoxic pattern on cell culture. Methodology. 3 carbon nanosphere materials were synthesized by sol-gel method. Particles shape, size and functionalization were assessed. Cytotoxic effect of synthesized materials was evaluated on SH-SY5Y cell line using XTT and crystal violet assays and imaging. Contribution. In this work we have demonstrated that cytotoxic effect of carbon nanospheres can be attenuated by varying the conditions of synthesis.

Resumen

Objetivo. Diferentes tipos de nanopartículas, incluidas las de carbono, han sido analizadas para una gran variedad de aplicaciones biológicas. Si bien, los resultados han sido mayoritariamente prometedores, se revelaron algunos efectos secundarios no deseados. La mayoría de los estudios biológicos se realizaron en nanoesferas de carbono altamente funcionalizadas, por lo que el objetivo principal de este estudio fue evaluar la citotoxicidad de las nanoesferas de carbono sin funcionalizar y sintetizadas por el método sol-gel. Inferimos que el material no funcionalizado presentará un patrón citotóxico en el cultivo celular. Metodología. Se sintetizaron 3 materiales de nanoesferas de carbono por el método sol-gel. Se evaluó la diferencia en propiedades como forma y tamaño. El efecto citotóxico de los materiales sintetizados se evaluó en la línea celular SH-SY5Y utilizando ensayos XTT y cristal violeta y por el análisis estructural de organelos. Contribución. Se demostró que el efecto citotóxico de las nanoesferas de carbono puede atenuarse variando las condiciones de síntesis.

Carbone nanosphere, Neurotoxicity, Sol-gel

Nanoesferas de carbono, Neurotoxicidad, Sol-gel

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Introduction

Different nanomaterials are widely investigated nowadays to be used for different biological applications, such as drug delivery, imaging, anticancer therapy, etc. Alongside with benefits, some materials present severe side effects: cells integrity disruption, metabolism modification, nuclei alterations among others.

Carbon nanoparticles (fullerenes, nanotubes and graphene) were promising materials for biological applications due to their biocompatibility, high surface area and chemical stability. However, in some cases they also present undesirable effects in biological studies. Carbon nanospheres are relatively new carbon nanometric-scaled material. Due to their high surface area and easiness to be functionalized and/or loaded with therapy agent, carbon nanospheres were extensively investigated. For instance, different carbon nanosphere materials were tested as imaging agents, drug delivery carriers, for phototherapy of cancer giving promising results. Nevertheless, some cytotoxic effects were disclosed for that materials too. It worth mention that almost in all studied cases, carbon nanoparticles were produced by hydrothermal synthesis, thus particles were functionalized with different hydrophilic groups. For that reason, our research group decided to assess cytotoxicity of unfunctionalized carbon nanospheres synthesized by sol-gel method. We hypothesize that unfunctionalized material will present different cytotoxic pattern on cell culture.

Methodology

Synthesis. For carbon nanospheres synthesis two-step method proposed by Liu et al was used. At the first step polymeric spheres were synthesized from resorcinol and formaldehyde under different temperatures and synthesis time. At the following step, polymeric materials were carbonized at 900 °C under nitrogen atmosphere for 3 h.

Resulted carbon materials were named SG-1, SG-2 and SG-3.

Characterization. All materials were characterized by scanning electron microscopy. RAMAN and FTIR spectra also were obtained for the materials.

Biological experiments. Neuroblastoma SH-SY5Y cell line was used to evaluate neurotoxic effect of carbon nanospheres. Cells were grown in Dulbecco modified Eagles minimal essential medium supplemented with 10% of fetal bovine serum until they reached confluency. SH-SY5Y were plated at 10^5 concentration into 96-well plate for 24 h. After that, cells were incubated with different concentrations of nanospheres separately for 24 h. XTT assay was performed using standard protocol. Crystal violet (CV) assay was performed on the same cell culture immediately after XTT assay. The results were normalized to control. For cytoskeleton and nucleus staining, cells were plated on microscopy crystals for 24 h prior the staining. The staining was performed with Phalloidin-FITC and DAPI.

Results

Synthesis and characterization.

Scanning microscopy images have shown that particles of three studied materials possess spherical shape with diameters 600-700 nm, approximately.

FTIR have not evidenced any functional group peaks, which is consistent with temperature of carbonization.

RAMAN spectra have demonstrated only D and G bands, giving crystallinity ratio of materials of 60-70%.

Biological studies.

Since XTT assay evaluate mitochondrial activity of cells and CV depicts total biomass, both tests should be performed in order to assess effect of nanoparticles on cell culture. 4 different concentrations of carbon nanospheres were implemented: 50, 100, 150 and 200 µg/mL.

XTT and CV assay results are shown at Graphic 1 and Graphic 2, respectively.

Graphic 1 Mitochondrial metabolic activity of SH-SY5Y exposed to carbon nanospheres

Source: kown work [Excel]

Graphic 2 Biomass of SH-SY5Y exposed to carbon nanospheres

Source: own work [Excel]

The results showed that the material SG-1 present greater dose dependent cytotoxic effect on neuronal cells, giving almost 50% cell activity reduction. Cytotoxic effect of SG-2 and SG-3 is more moderate, with approximately 10% of mitochondrial activity reduction. We suppose that these differences may be caused by different surface geometry, such as pore size and its distribution, that resulted from distinct synthesis conditions. It is known that larger porosity leads to higher surface area, which may cause better interaction between particle and cell membrane.

This effect cannot be attributed to different cell mass, since CV assay did not show it decline, but rather it increases in some extend. For better understanding of particle-cell interactions, staining of cells after their exposure to the materials was performed (Figure 1).

The images showed that SG-1 is more damaging for both cytoskeleton and nucleus, meanwhile, SG-3 did not present significant differences comparing to control.

Figure 1 Cytoskeleton (FITC) and nucleus (DAPI) images

Source: own work [PowerPoint]

Conclusions

Neurotoxic effect of unfunctionalized carbon nanosphere materials such as loss of cytoskeleton integrity and nuclei damage can be attenuated by different conditions of synthesis.

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