

## Short Communication

Nanostructured Pt(NH<sub>3</sub>)<sub>4</sub>Cl<sub>2</sub>/SiO<sub>2</sub> for nanomedicine: catalytic degradation of DNA in cancer cellsTessy López<sup>1,2,3</sup>, Emma Ortíz Islas<sup>2</sup>, Mayra A. Alvarez Lemus<sup>2\*</sup> and Richard Donald González<sup>3</sup><sup>1</sup>Health Department, Universidad Autónoma Metropolitana-Xochimilco, México City, México;<sup>2</sup>Nanotechnology Laboratory, National Institute of Neurology and Neurosurgery, México City, México;<sup>3</sup>Department of Chemical and Biomolecular Engineering, Tulane University, New Orleans, LA, USA

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## Abstract

*In vivo* suppression of glioblastoma multiforme (GBM) in Wistar rats using silica-shelled biocatalytic Pt(NH<sub>3</sub>)<sub>4</sub>Cl<sub>2</sub> nanoparticles is reported. These nanoparticles were synthesized by a sol-gel technique and characterized by SEM and HRTEM imaging. We confirmed morphological uniformity (30 nm) and surface acidity of the nanoparticles, respectively, by TEM imaging and FTIR spectral analysis. Interestingly, treatment of Wistar rats intraperitoneally inoculated with C<sub>6</sub> cells using the biocatalysts resulted in considerable tumor shrinkage. Efficiency of the biocatalyst to shrink a tumor is superior to that by the commercial cytotoxic agent cisplatin. The tumor suppression property of Pt(NH<sub>3</sub>)<sub>4</sub>Cl<sub>2</sub> nanoparticles is attributed to catalytic damage of DNA in C<sub>6</sub> cells.

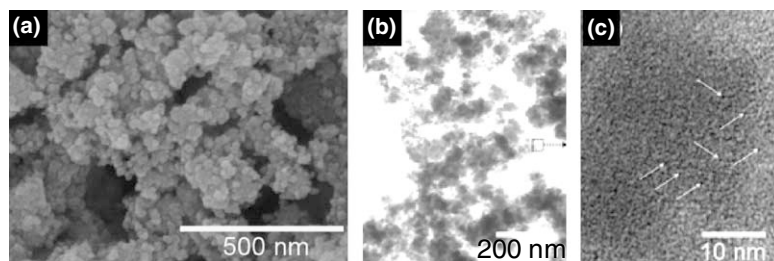
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Controlled drug delivery to the brain date back to the 1970s, when Chisholm and Singer (1) used a cannula to release fluids directly into the central nervous system (CNS) of rats without any perceptible harm to the animal. These studies generated great interest in the administration of drugs with microinjections in specific areas (2–5). Recently, the use of nanoparticles for cancer treatment has attracted much attention, mainly in the drug delivery (6) and photodynamic therapy fields (7). Doxorubicine, BCNU (8), methotrexate, and 5-FU are chemotherapeutic agents that are extensively encapsulated in a great variety of nanoparticles.

Due to the blood brain barrier (BBB), only a small number of chemotherapeutic agents are suitable for the treatment of brain tumors (9). Currently Temozolomide is the best well-tolerated drug that can cross BBB (10–13). In the case of malignant tumors like glioblastoma multiforme (GBM) (13–16), Temozolomide can improve life expectancy by 6 months. Because GBM spreads very

rapidly and infiltrates healthy tissue, complete resection is practically impossible, leaving the survival index of patients with GBM rather low even after surgery, radiotherapy, and chemotherapy.

Due to its well-known DNA reactivity, cisplatin exhibits high antitumoral activity and is one of the most widely accepted chemotherapeutic agent in the treatment of several cancers. However, some recent reports show that only cis-conformations of platinum complexes are effective against cancer (17, 18). Here we report *in vivo* suppression of GBM in Wistar rats treated with silica-shelled tetraminedichloro platinum(II) nanoparticles. These nanoparticles were prepared by incorporating tetraminedichloro platinum(II) in silica shells by a sol-gel process. We investigated relations among acidic sites on the nanoparticles, *in vivo* interactions between Pt-supported nanoparticles and DNA in C<sub>6</sub> cells, and tumor suppression.



**Fig. 1.** (a) Scanning electron micrograph and (b and c) TEM images of  $\text{Pt}(\text{NH}_3)_4\text{Cl}_2/\text{SiO}_2$  nanoparticles. Arrows in (c) indicate  $\text{Pt}(\text{NH}_3)_4\text{Cl}_2$  complexes embedded in silica shells.

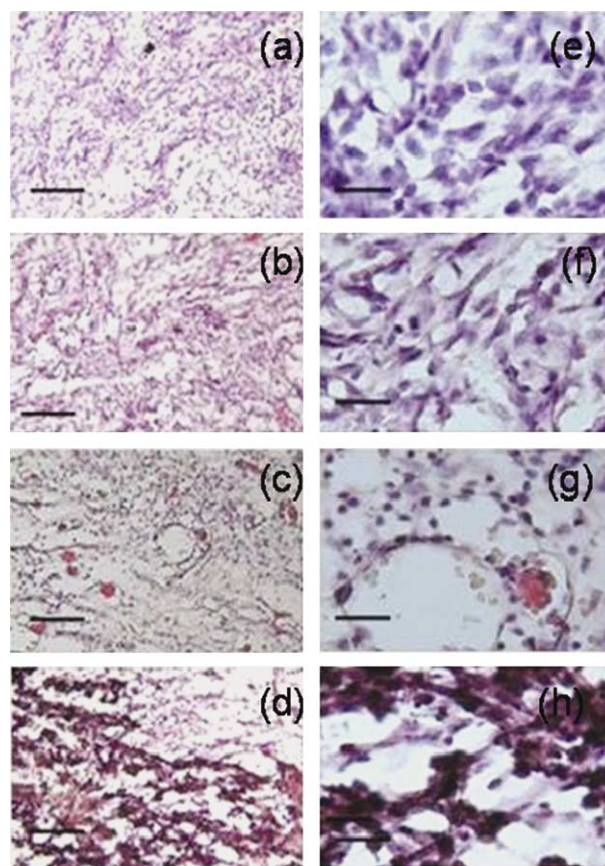
## Results and discussion

We examined the morphology and structure of  $\text{Pt}(\text{NH}_3)_4\text{Cl}_2/\text{SiO}_2$  nanoparticles by SEM and TEM imaging and energy dispersive spectroscopy (EDS). A typical SEM image of  $\text{Pt}(\text{NH}_3)_4\text{Cl}_2/\text{SiO}_2$  nanoparticles is shown in Fig. 1a. Morphology of the material indicates agglomeration of small particles into non-uniform nanostructures. High resolution TEM image provided us with evidence for  $<1$  nm diameter  $\text{Pt}(\text{NH}_3)_4\text{Cl}_2$  complexes embedded in silica shells (Fig. 1c). These nanoparticles were further characterized by an EDS analysis, which indicates the presence of silicon, oxygen, platinum, and chlorine (supplementary information).

We investigated *in vivo* suppression of  $\text{C}_6$  tumor in a Wistar rat using the biocatalytic  $\text{Pt}(\text{NH}_3)_4\text{Cl}_2/\text{SiO}_2$  nanoparticles. At first,  $\text{C}_6$  cells (obtained from the American Tissue Culture Collection, Rockville, MD) were cultured into sufficient quantity and, subsequently,  $1 \times 10^7$   $\text{C}_6$  cells were intraperitoneally inoculated in a Wistar strain male rat in order to develop a GBM type tumor. Twenty days after inoculation the  $\text{C}_6$  tumor had grown to an acceptable size in 80% of the animals (19). When the rats developed a tumor of 2 cm, they were randomly allocated into four groups as follows: (A) control (no administration), (B) administered with a suspension of the Pt complex, (C) administered with a suspension of sol-gel silica nanoparticles, and (D) administered with a suspension of  $\text{Pt}(\text{NH}_3)_4\text{Cl}_2/\text{SiO}_2$  nanoparticles. Twenty-one days after the administration of these nanoparticles, tumors were surgically extracted and analyzed *ex vivo*. Also, we examined the effect of *in vivo* administered nanoparticles on tumor size. We found that suppression of the tumor was negligible in the group of rats administered with the Pt complex or sol-gel silica nanoparticles. On the other hand, a decrease in the tumor size (approximately a 73% reduction) was significant in the group of rats administered with sol-gel  $\text{Pt}(\text{NH}_3)_4\text{Cl}_2/\text{SiO}_2$  nanoparticles. We attribute that the tumor suppression activity of sol-gel  $\text{Pt}(\text{NH}_3)_4\text{Cl}_2/\text{SiO}_2$  nanoparticles comes from killing of cancer cells via free radicals inducing DNA damage. In other words, the biocatalytic nanoparticles were possibly taken up by cancer cells and free radical centers on the surface of the nanoparticles

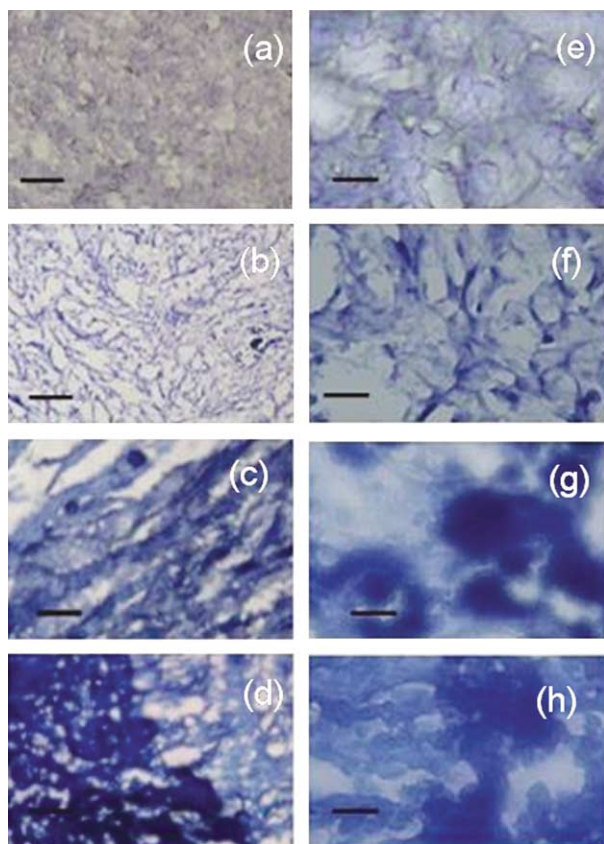
inducing cell death via DNA damage. These possibilities investigated by *ex vivo* analysis of tumor sections and *ex vivo* and *in vitro* analyses of DNA damage in  $\text{C}_6$  cancer cells.

The H-E micrographs of histological sections from tumors after treatment with  $\text{Pt}(\text{NH}_3)_4\text{Cl}_2/\text{SiO}_2$  nanoparticles are shown in Fig. 2. We analyzed different tumor regions at low and high magnifications. Histological studies were conducted following the path of injection to associate direct action of nanoparticles with tumor suppression. Fig. 2b shows the area in H-E stained sections from the  $\text{C}_6$  tumor with viable tumor cells where



**Fig. 2.** H-E stained sections from the  $\text{C}_6$  tumor in a Wistar rat treated with  $\text{Pt}(\text{NH}_3)_4\text{Cl}_2/\text{SiO}_2$ . (a–c) Low magnification and (d–h) high magnification.





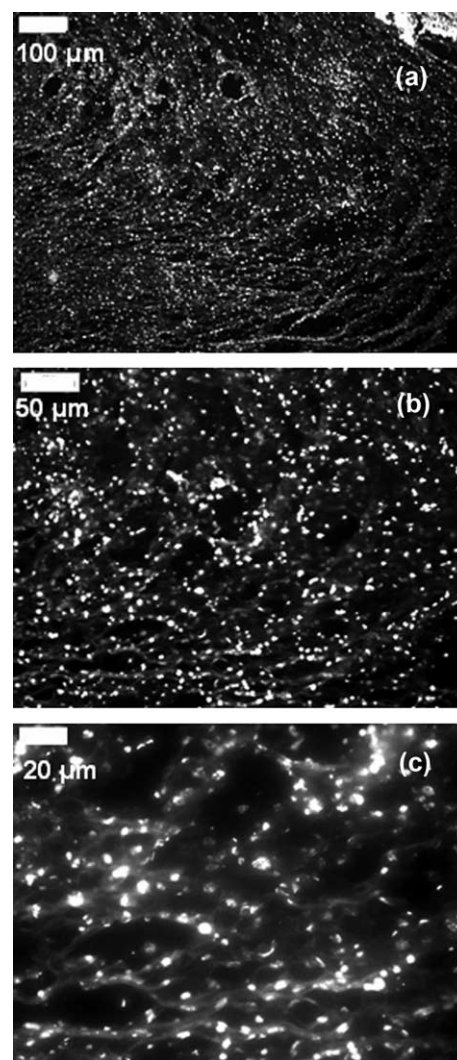
**Fig. 3.** Trypan blue histological images of sections from  $C_6$  tumors in Wistar rats treated with  $Pt(NH_3)_4Cl_2/SiO_2$  nanoparticles. (a–c) Low magnification and (d–h) high magnification.

characteristic GBM cells morphology can be identified. In Fig. 2d, two different zones are observed: the upper region consists more of viable tumor cells; whereas, cell damage is remarkable in the lower region. The tumor suppression by  $Pt(NH_3)_4Cl_2/SiO_2$  nanoparticles is clearly presented at a higher magnification in Fig. 2h, which is indicative of a chemotherapeutic effect of the nanoparticles. On the other hand, we could not identify any remarkable changes to tumors in control animals (group A–C).

The *in vivo* effect of  $Pt(NH_3)_4Cl_2/SiO_2$  nanoparticles on the  $C_6$  tumor was examined by the cell viability assay of an extracted tumor using Trypan blue. In this assay, live cells exclude Trypan blue dye by retarding its intracellular diffusion; whereas, the dye effectively enters inside dead cells because of their poor cell wall integrity. Fig. 3 shows histological images of a  $C_6$  tumor *in vivo* treated with  $Pt(NH_3)_4Cl_2/SiO_2$  nanoparticles. Remarkable intensity of the stain is indicative of anticancer activity of the nanoparticles. Dead cells in the  $C_6$  tumor are clearly identified in images at higher magnification (Fig. 3c–h). We assume that the anticancer activity of  $Pt(NH_3)_4Cl_2/SiO_2$

$SiO_2$  nanoparticles originates as a result of intracellular uptake of the nanoparticles, interactions of the nanoparticles with genetic materials, and subsequent damage of DNA.

We examined DNA damage for  $C_6$  cells in tumors treated with the biocatalytic nanoparticles by analyzing terminal transferase (TUNEL assay). In the TUNEL assay, end labeling techniques are employed for studying the actual mechanism of DNA fragmentation, as well as the detection and characterization of endonucleases. Endonucleases cleave DNA by attacking the phosphodiester bonds of the sugar–phosphate backbone of each strand. The phosphodiester bond can be cleaved in two ways such that the phosphate is left on either the 3' end of the DNA strand or the 5' end, the opposite end being left with a hydroxyl group in each case. The TUNEL image of a  $C_6$  tumor is shown in Fig. 4. The white dots observed in



**Fig. 4.** Representative TUNEL images from a  $C_6$  tumor: (a) Tumor treated using  $Pt(NH_3)_4Cl_2/SiO_2$  and (b–c) higher magnification.

Fig. 4 represent fluorescent ends of fragmented DNA. The TUNEL technique is widely used to quantify apoptotic cells, because it allows identifying affected cells. This technique detects DNA fragmentation by endogenous DNA, the final step in apoptosis. In order to identify the cell death process (apoptosis or necrosis), a specific fluorescent enzyme is used to mark the ends of the fragmented DNA. This is characteristic of cell death by apoptosis (programmed cell death), instead of necrosis in which the membrane collapses and cells are rapidly destroyed. Therefore, the white dots in Fig. 4 is indicative of DNA fragmentation in tumors treated with  $\text{Pt}(\text{NH}_3)_4\text{Cl}_2/\text{SiO}_2$  nanoparticles. The effect of  $\text{Pt}(\text{NH}_3)_4\text{Cl}_2/\text{SiO}_2$  nanoparticles on DNA damage and fragmentation was confirmed from *in vitro* treatment of  $\text{C}_6$  cells with the nanoparticles (supplementary information).

In summary, we developed a new biocatalytic nanoparticle for the treatment of tumors. Wistar rats intraperitoneally inoculated with  $\text{C}_6$  cancer cells and treated with the biocatalyst resulted in nearly 73% shrinkage of the tumor. The tumor suppression is due to the killing of cancer cells via intracellular uptake and DNA fragmentation. The small dimension of the  $\text{SiO}_2$  particles along with  $\text{Pt}(\text{NH}_3)_4\text{Cl}_2$  enable intracellular uptake of the nanoparticles. Also, the uniform porous nature of supported  $\text{Pt}(\text{NH}_3)_4\text{Cl}_2/\text{SiO}_2$  with small particle size (1–2 nm) is likely the prerequisite for an efficient catalytic degradation of DNA in cancer cells. These results suggest that administration of chemotherapeutic nanoparticles can be an effective treatment for aggressive tumors such as GBM. We firmly believe that a new chapter in catalytic nanomedicine has been opened in the chemotherapeutic treatment of malignant tumors through the use of biocatalytic nanoparticles.

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## Conflict of interest and funding

There is no conflict of interest in the present study for any of the authors.

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