Multifunctional Mesoporous Silica Nanoparticles for the Delivery of Cisplatin and Gemcitabine to Treat Cancer

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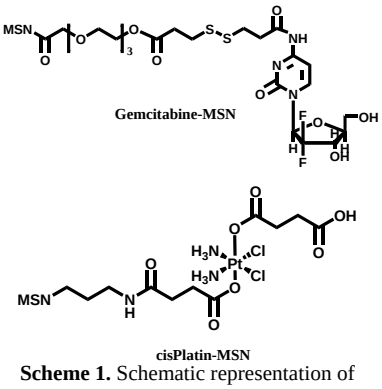
Statement of Purpose: Cancer is a challenging public health issue in the United States and many other parts of the world. Currently, one in four deaths in the United States is due to cancer. Despite remarkable efforts spent on drug research and development, there has not been a significant increase in overall patient survival for many types of cancer. The treatment possibilities for many cancers have remained nearly unchanged for decades as a result of the lack of new drug approvals. The currently approved chemotherapeutic agents, mostly small molecules, are limited by their nonspecific biodistribution and poor pharmacokinetics, leading to dose-limiting side effects. There exists a critical need to identify approaches to make small molecule drugs more bioavailable and increase tumor uptake of the agent while minimizing nonspecific uptake. Nanoparticle-based drug delivery systems are an emerging class of platforms that have the potential to revolutionize cancer chemotherapy. A variety of different nanocarriers have been developed to deliver a wide range of cancer chemotherapeutics. Mesoporous silica nanoparticles (MSNs) have attracted much attention for their potential application in this field. Their ability to carry large payloads and tunable diffusional release of drug molecules gives rise to a biogenic local concentration at the targeted area, which reduces the overall dosage and prevents any acute or chronic complications. The main goal of this project is to develop multifunctional MSNs for the target-specific delivery of anticancer agents. In particular, we are interested in cisplatin and gemcitabine, two widely use and FDA approved chemotherapeutic agents. The platform consists of cisplatin or gemcitabine pro-drugs chemically attached to MSNs through stimuli-responsive linkers (**Scheme 1**). These linkers are broken releasing the anticancer agent under reducing conditions such as those found in cancer cells. These materials can be further functionalized with poly(ethylene glycol) (PEG) chains and targeting agents to improve their therapeutic performance *in vitro and in vivo*.



Scheme 1. Schematic representation of MSN-based drug delivery systems developed in this project.

Methods: *Synthesis of gemcitabine prodrug.* The synthesis of the gemcitabine prodrug was carried out through a multi steps approach. All the steps in this synthesis were characterized by ^{1}H and ^{13}CNMR, IR and ESI-MS. *Synthesis of cisplatin prodrug.* The synthesis of the cisplatin prodrug was carried out in two steps. Cisplatin is readily oxidized by hydrogen peroxide to produce *cis, cis, trans*-diamminedichlorodihydroxy platinum(IV). This Pt(IV) complex was reacted with succinic anhydride to afford the corresponding carboxylated complex. Both steps in the synthesis of the cisplatin prodrug were characterized by ^{1}H and ^{13}CNMR, IR and ESI-MS. *Synthesis and functionalization of aminopropyl-MSNs (AP-MSNs).* The synthesis of AP-MSNs was carried out by surfactant-templated co-condensation approach. Cetyltrimethylammonium bromide (CTAB) and tetraorthosilicate (TEOS) were used as surfactant and silica precursor, respectively. CTAB was washed out from the MSN material under acidic conditions. AP-MSNs were further functionalized with either cisplatin or gemcitabine prodrug. Additionally, cisplatin-MSN material was functionalized with PEG polymer to enhance the colloidal stability. The nanoparticles were characterized by dynamic light scattering (DLS), Z-potential, thermogravimetric analysis (TGA), and scanning and transmission electron microscopy (SEM & TEM). *Cytotoxicity.* HeLa cells were seeded in six-well plates with a density of $1x10^{5}$ cells/mL in 3 mL of cell medium and incubated at 37 °C with a 5% CO_2 atmosphere for 24 h. Then, the cells were inoculated with different materials, AP-MSNs, cisPt-MSNs, PEG-cisPt-MSNs and Gem-MSNs at concentrations of 1, 5, 20, 50 and 100 μg/mL for other 48 h. After the incubation each well was washed with PBS and the cells were trypsinized, centrifuged, and re-suspended in cell medium. Viability was determined by the trypan exclusion assay.

Results: MSN materials were synthesized and functionalized with cisplatin or gemcitabine prodrug. The structural characterization shows polydispersed nanoparticles (150-250 nm) with neutral charge after being functionalized with the stimuli-responsive linker and PEG chain. The therapeutic properties of these materials were tested *in vitro* using HeLa cells. Both materials, cisPt-MSNs and Gem-MSNs, present a high cytotoxicity (**Figure 1**). The PEG-cisPt-MSNs showed an enhance toxicity, presumably due to better colloidal stability.

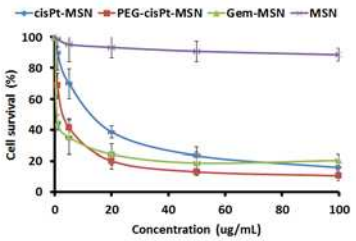


Figure 1. Cytotoxicity of the prodrug-loaded MSNs materials.

Conclusions: We successfully synthesized stimuli-responsive MSN materials containing either cisplatin or gemcitabine prodrug. These drug delivery platforms showed effective therapeutic effect against HeLa cells. Further functionalization with targeting agents will enhance the target capability of these systems.