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The design of core-shell (gold core mesoporous organosilica shell) nanoparticles from two molecular precursors bis(triethoxysilylpropyl)tetrasulfide and bis(triethoxysilyl)ethane is described. The precursors bring ordered mesoporosity when combined with cationic surfactant cetyltrimethylammonium bromide during the sol-gel procedure while bis(triethoxysilylpropyl)tetrasulfide brings biodegradability to the structure. The molecular precursors allowed constructing small core-shell nanoparticles of 280 nm diameter compatible with the endocytosis of the nanoparticles by cancer cells. A functional ordered mesoporosity was obtained. Indeed, the interactions of gemcitabine monophosphate with the pores made by the molecular precursors allowed encapsulating a high amount of this drug (35% weight). The drug was not released at physiologic pH, but led to a high cancer cell killing effect of 60%. The highly functionalized nanoparticles we designed allowed performing two-photon imaging of cancer cells thanks to the gold core of the nanoparticles. Combined with gemcitabine monophosphate delivery, the nanoparticles we propose could lead to a very precise treatment of cancers in the future, with reduced side-effects and enhanced efficiency. Therefore, these nanoparticles are very promising for theranostics and nanomedicine applications.
Gold Core Mesoporous Organosilica Shell Degradable Nanoparticles for Two-Photon Imaging and Gemcitabine Monophosphate Delivery.

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The synthesis of gold core degradable mesoporous organosilica shell nanoparticles is described. The nanoparticles were very efficient for two-photon luminescence imaging of cancer cells and for in vitro gemcitabine monophosphate delivery, allowing promising theranostic applications in the nanomedicine field.

Introduction
Organosilica nanoparticles have attracted much attention for biological applications in the last decade and the field has been recently reviewed. The organic part in these nanoparticles represents 80-90% of the structure leading thus to unique features which makes them very suitable for theranostic applications. Furthermore, porous systems have been reported which allow loading drugs such as doxorubicin with a very high efficiency. However, these nanoparticles are very stable in simulated biological fluids which could be problematic for their elimination and clearance. Therefore, combining degradability with organosilica nanomaterials would be of high interest to avoid accumulation of these nanoparticles in the body with elimination through renal clearance, while providing excellent loading capacities and targeting properties for cancer applications. We and others have recently described mesoporous organosilica nanostructures incorporating disulfide and tetrathiol bridges which are degraded by glutathione reduction. For imaging applications, two-photon excitation presents attractive features such as high spatial resolution with 3D reconstruction, low scattering losses, low autofluorescence and high depth penetration in tissues. Gold nanoparticles are particularly suitable for two-photon excited luminescence and their penetration in 3D spheroids has been studied using this technique. Following our work on gold core-periodic mesoporous organosilica shell nanoparticles for two-photon excitation, we decided to investigate gold core degradable mesoporous organosilica shell nanoparticles based on tetrathiol bridges for two-photon imaging of cancer cells and drug delivery. We decided to study a highly hydrophilic drug: gemcitabine monophosphate (GMP). Nanoparticle formulation with GMP is very challenging, covalent systems such as squalenization, formulations with ion coordination (MOF, or Gd), and calcium phosphate-based systems have been therefore developed. We anticipated that the interactions of GMP with the organosilica matrix would stabilize the complex allowing an efficient delivery of this drug in cancer cells. Herein, we report the first synthesis of degradable core shell nanoparticles Au@BTSE-BTSPS, BTSE: 1,2-bis(triethoxysilyl)ethane BTSPS bis[3-(triethoxysilyl)propyl] tetrathiol composed of gold nanoparticles as the core and the degradable tetrathiol-based organosilica as the shell. The luminescence of the core allowed tracking the nanoparticles in cancer cells with two-photon excitation and the porous shell allowed loading GMP with a very high efficiency and the delivery of GMP in cancer cells.
Results and Discussion:

The nanoparticles (Au@E-TS), composed of 13 nm gold cores inside 280 nm organosilica spheres, were synthesized in two steps. At first, gold nanoparticles were prepared using the Frens method. They were then stabilized with APTES in order to incorporate them in a micellar solution of CTAB, and to subsequently perform the hydrolysis polycondensation of the organosilane precursors. The co-condensation of 1,2-bis(triethoxysilyl)ethane (E) and bis[3-(triethoxysilyl)propyl] tetrasulfide (TS) precursors (Figure 1) was thus performed at 50°C for 2 h in mild conditions.

Figure 2. TEM images of Au@E-TS NPs (A, B). N_{2}-adsorption-desorption isotherm (C). UV-vis spectra of the Au NPs and Au@E-TS NPs (D).

Figure 3. Two photon luminescence imaging on living cells. MDA-MB-231 expressing GFP and incubated or not with Au@E-TS at 80 µg.mL^{-1} for 24 h. Membranes of cells were stained with orange cell mask and imaging was performed at 561 nm. Nuclei expressing GFP were visualized at 488 nm and Au@E-TS were excited with two-
The core-shell nanoparticles were isolated though centrifugation and the surfactant was removed from the pores using an ammonium nitrate 95% EtOH solution. Au@E-TS nanoparticles were then characterized via transmission electron microscopy (TEM), which depicted spherical core shell NPs composed of several gold cores inside porous organosilica spheres with diameter ranging from 250 to 300 nm (Fig 2.A-B). The distribution of the NPs confirmed the nanoscale size of the carriers (Fig S2). Besides, as shown by the N\textsubscript{2} adsorption desorption analysis, nanoparticles present a specific surface areas of 413 m\textsuperscript{2}.g\textsuperscript{-1} (fig 2.C). The optical properties of Au and Au@E-TS NPs were then analyzed. By comparing the UV-visible absorption spectrum of Au@E-TS NPs with the UV-vis spectra of Au NPs (Fig 2.D), an important red shift with an important enlargement of the plasmon band was observed due to the aggregation of the gold NPs core inside the organosilica shell. The morphology of the core-shell system was investigated by using dark-field scanning TEM (DF-STEM) (fig S3A), DF-STEM mapping (Fig S3 B-E) whereas the gold core crystal structure of Au@E-TS NPs was studied with electron diffraction (Fig S3F). The distribution of the elements in NPs was studied with DF-STEM and electron energy-loss spectroscopy (Fig S3 B-E). A homogeneous distribution of oxygen, carbon, sulfur and silicon atoms was observed which shows that no phase demixing occurred during the sol-gel procedure. This verification is particularly important as the efficient degradation of the shell depends on the homogenous dispersion of sulfur within the NPs matrix. X-ray diffraction (XRD) patterns in Figure S4.A of Au@E-TS nanoparticles at small angles displayed the presence of the peaks at 2.03° corresponding to regular repetitions of mesopores. Besides, the wide angle X-ray diffraction pattern in Figure S4.B showed a broad peak at 23°, which corresponds to non-regular repetitions within the siloxane framework, with two peaks at 37.4° and 43.5° which corresponds to gold metal.

After checking the morphology and the composition of Au@E-TS NPs, we then investigated their biocompatibility as well as their capabilities for two-photon excitation excited confocal luminescence imaging of cancer cells. Before the imaging experiments, MDA-MB-231 cancer cells were washed twice, then Au@E-TS NPs were incubated in cancer cells for 24 hours at 80 µg mL\textsuperscript{-1} and the cell membranes were stained with Cell Mask Orange for 15 minutes at 1 µL mL\textsuperscript{-1} before the imaging experiments. As shown in Figure 3, the intra cellular two-photon excited fluorescence luminescence of the nanoparticles confirmed the successful endocytosis of the nanoparticles. Note that, the presence of gold as a core in the NPs contributed to the detection of Au@E-TS, showing that these nanocarriers were efficient for TPE, probably due to the aggregation of the gold NPs into the organosilica matrix thus leading to a plasmon resonance in the NIR.\textsuperscript{27} After having shown the endocytosis of Au@E-TS NPs, we then examined the biodegradable core shell NPs as vectors for GMP delivery. The loading of these nanoparticles with GMP was examined in water at room temperature. The loading capacity was obtained by UV-Vis absorption spectra of the supernatants and was 35 wt %. After that, the release of the drugs was then examined. Drug release experiments were first carried out at pH 7 in ultrapure water, with GMP loaded nanoparticles. No release of the drug was observed in these conditions showing the important interactions between the drug and the organosilica matrix. The release of GMP was shown when the pH was adjusted to 5.5 (lyosomal pH, Fig S5). The delivery of GMP was tested in MDA-MB-231 cancer cells (Fig.4). No significant cytotoxicity of empty nanoparticles was observed up to a concentration of 50 µg.mL\textsuperscript{-1} showing the biocompatibility of the biodegradable core shell NPs. Furthermore, the GMP delivery and cancer killing was highly efficient, with down to 40% of cell survival at only 10 µg.mL\textsuperscript{-1} of nanomaterials.

**Conclusions**

In summary, we have designed gold core shell mixed organosilica nanoparticles by the co-condensation of bis-(triethoxysilyl) ethane and the bis (3-(triethoxysilyl) propyl)tetrasulfide. The morphology and compositions were fully characterized by various techniques. These nanoparticles were tested in vitro with MDA-MB-231 cancer cells for two-photon luminescence imaging and for gemcitabine monophosphate delivery showing promising potential for biomedical applications.

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Figure 4. GMP delivery in MDA-MB-231 cancer cells with Au@E-TS NPs. Cytotoxic study of a range (from 0.1 to 50 µg mL⁻¹) Au@E-TS NPs incubated for 72 h with MDA-MB-231 cancer cells.

Notes and References

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