Electronic Supplementary Information

Gold Nanocage-CNT Hybrid for Targeted Imaging and Photothermal Destruction of Cancer

Sadia Afrin Khan, Rajashekhar Kanchanapally, Zhen Fan, Lule Beqa, Anant Kumar Singh, Dulal Senapati, and Paresh Chandra Ray *

Department of Chemistry and Biochemistry, Jackson State University, Jackson, MS, USA; E mail: paresh.c.ray@jsums.edu; Fax:+16019793674

Content

Materials and Experiments: Hydrogen tetrachloroaurate ($HAuCl_4.3H_2O$), $NaBH_4$, sodium citrate, ATP, silver nitrate were purchased from Sigma-Aldrich and used without further purification. 3'-SH and 5'-Cy3 modified A9 RNA aptamer (5'-Cy3 -GGG AGG ACG AUG CGG ACC GAA AAA GAC CUG ACU UCU AUA CUA AGU CUA CGU UCC CAG ACG ACU CGC CCG 3'–SH were purchased from Midland Certified Reagent. The human prostate cancer cell line LNCaP which over expresses a high level of prostate-specific membrane antigen (PSMA), was obtained from the American Type Culture Collection (ATCC, Rockville, MD). PSMA negative human prostate cancer cell line (PC-3) [24-25] was also purchased from ATCC. Human skin HaCaT keratinocytes, a transformed human epidermal cell line, was obtained from Dr. Norbert Fusenig of the Germany Cancer Research Center, Heidelberg, Germany.

Synthesis of gold nanocage decorated SWCNT hybrid material: For the chemical design of hybrid nanomaterial, at first we have synthesized the gold nanocage using two-step procedure as reported before [17-18]. In the first step, we have synthesized silver nanocube. For this purpose, 18ml of ethylene glycol was heated at 165^0C for 1 hr with continuous stirring. Then 240 µl of Na_2S (3mM), 4.5 ml PVP, 1.5 ml $AgNO_3$ (282mM) were sequentially added. We continued the heating up to 15 min until the color changed to yellowish green, which indicates the formation of silver nanocube. In the second step, we have made gold nanocage from silver nanocube using galvanic replacement reaction. For this purpose, we dissolved 1 ml of concentrated Ag nanocube in 50 ml nano pure water and then increased the solution temperature up to boiling point. After that, we added different volumes of 10^{-3} M HAuCl4. 3 H_2O solution to prepare gold nanocage, as shown in Figure 2A. Next, SWCNTs were attached to gold nanocage through para-aminothiophenol, through several step processes as shown in Figure 1. In the first step, chemical functionalization of SWCNT tips was performed mainly by using oxidative treatments using 3:1 ratio of concentrated sulfuric acid and nitric acid [8,11,20-23]. As shown in Figure 1, oxidation process yields opened tubes with carboxylic acid functionality at both the sidewall and the tube endings[8,11,20-23]. In second step, acid chloride functionalized SWCNTs were prepared by treating –COOH functionalized SWCNTs with thionyl chloride in presence of DMF catalyst under inert

medium. In the third step, the acid chloride group was used as chemical anchors for further derivatization with para-aminothiophenol (ATP), through which CNT will be conjugated with gold nanocage via thiol group, as described in Figure 1. Since free thiol group in ATP attached SWCNT is available for linkage with gold nanoparticle, in the fourth step gold nanocage was attached with SWCNT using –SH linkage via aminothiophenol, as shown in Figure 1. After functionalization, free gold nanoparticles were separated after centrifuging the mixture at 4,000 rpm for 30 minutes. As shown in Figure 2B our TEM image data clearly show that the gold nanocages are nicely decorated on SWCNTs. Figure 2D shows the absorption spectra of only SWCNT, gold nanocage and hybrid material. Broad and structureless absorption spectrum from near infra-visible regions from 400 to 1200 nm is mainly due to the E_{11} and E_{22} transitions of nanotubes [20-23]. As shown in Figure 2D, in case of gold nanocage, we observed a strong long wavelength plasmon band around 800 nm, which is due to the oscillation of the conduction band electrons [20-23]**.** When we attached gold nanocage with SWCNT, our experimental results show that the absorption spectum shifts toward higher wavelength and becomes broad. And it is mainly due to the fact that gold nanocages are very closely packed on SWCNT as shown in Figure 2B.

Synthesis of A9 RNA aptamer conjugated hybrid material: -SH modifed aptamers were gradually exposed to gold nanocage decorated SWCNTs in the presence of 0.1 M NaCl in a PBS buffer over a 16-hour period. To remove the unbound aptamers, we centrifuged the solution at 8,000 rpm for 20 minutes and the precipitate was re-dispersed in 2 mL of the buffer solution. We have continued this process three times. To measure the number of aptamer molecules in each gold nanocage, we have performed fluorescence analyses, as we reported before [5,10-11]. We estimated that there were about 150-200 aptamers per one cage.

Fluorescence Imaging Analysis: Cancer cells were plated at a density of 10^5cells/well and incubated for 24 h for cell attachment. After that, the cells were incubated for 1-4 h with Cy3 modified A9 RNA aptamers attached gold nanocage modified SWCNT. Then they were washed three times to remove unbound nanomaterial and a fresh cell culture medium was added. For the fluorescence imaging, we have used an Olympus IX71 inverted confocal fluorescence microscope fitted with SPOT Insight Digital Camera. To decrease the autofluorescence signal, 488 nm light was selected for the excitation.

Flow Cytometry Analysis: Cancer cells on 24-well tissue culture plates were treated with Cy3 modified A9 RNA aptamers attached nanoparticle. Flow cytometry analyses were performed at 4 hours duration, using a BD Bioscience FACSCalibur equipped with a 488 nm argon laser. Mean fluorescence signals were averaged over all cells analyzed in 5 second intervals. For measuring the % of labeled cells, we have used only A9 RNA aptamers attached nanoparticle without dye as reference.

Cell Culture and Cellular Incubation with Nanoparticle: Cancer cells were grown in a 5% CO_2 incubator at 37 °C using RPMI-1640 medium (ATCC, Rockville, MD) supplemented with 10% premium fetal bovine serum (FBS) (Lonza, Walkersville, MD) and antibiotics (10 IU/mL penicillin G and streptomycin) in 75-cm^2 tissue culture flasks. An enzyme-linked immunosorbent assay kit was used to quantify PSMA in different tested cells.

Photothermal Therapy and percentage of live cell determination: For photothermal therapy experiment, we have used(1-2) W/cm^2 power 1064 nm NIR light for 10 minutes using a CW Nd:YAG laser. After that, we have used MTT test to find the amount of live cells during nanotherapy process. For this purpose, prostate cancer cells were seeded in 96-well plates (well diameter 6.4 mm) with a density of 100,000cells/well and allowed to attach for 24 h at 37 °C in a 5% CO_2 incubator, before the treatment. After incubation with 3 nM concentration for two hours, the cell monolayer in the wells was repeatedly rinsed with PBS buffer to remove the nonspecifically adsorbed nanomaterials remaining in the medium and then was exposed to the laser at 1064 nm. Cell viability was also determined 1 h after the photothermal treatment using the 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell proliferation assay kit (ATCC CA# 30-1010k). This experiment has been performed 5-6 times and the average values are reported in this manuscript.

Infrared Analysis: Infrared spectroscopy (IR) was used for characterization of chemical bonds before and after modifications if new bonds were formed. Transmittance spectra were obtained using a Nicolet Nexus 670 FT-IR equipped with a DTGS detector. Figure 2C shows the FTIR spectra of ATP modified SWCNT. Our FTIR spectrum clearly shows all the characteristic peaks for ATP [11,20-23]. The absorption peaks for -SH, -CS and –CN stretching vibrations are at 2524, 1088 and 1306 cm^{-1} respectively. The absorption peak at 3448 cm^{-1} peak is due to -NH asymmetrical stretching vibration and the one at 3359 cm^{-1} is the symmetrical stretching vibration of -NH. All the other peaks are mainly due to the SWCNT, -C=O and –C=C- stretching vibration bands of ATP.