Electronic Supplementary Information

Targeted highly sensitive detection/eradication of multi-drug resistant *Salmonella DT104* through gold nanoparticle-SWCNT bioconjugated nanohybrids

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Content

Materials and Experiments

Hydrogen tetrachloroaurate ($HAuCl_4$ x $3H_2O$), tri-sodium citrate (TSC), and single-walled carbon nanotubes (SWCNTs) were purchased from Sigma-Aldrich and used without further purification. Nanopure H_2O was purchased from Fisher Scientific. Ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline antibiotic, multiple drug resistant (MDR) *Salmonella typhimurium DT104*, and other bacteria strains were obtained from the American Type Culture Collection (ATCC, Rockville, MD; ATCC 700408). Antibody specific for *Salmonella DT104* were purchased from Abcam (Ab69238), and growth media for the bacteria were obtained from ATCC.

Functionalization of SWCNTs

200 mg SWCNTs, 2 g of DABCO, and 2 g of ethyl nitroacetate were dispersed in 20 mL of EtOH solvent by sonication for 10 min, and the system was placed under microwave reaction conditions at 130 ^oC for 2 hours, which afforded the corresponding isoxazoline functionalized SWCNTs. The product was washed with nanopure H_2O, EtOH and acetone. As a result of the cycloaddition, the ester functionality on isoxazoline ring could be utilized for further functionalization with the amine group from *p*-aminothiophenol, which also offers a thiol group for attaching with gold. In this step, 200 mg functionalized SWCNTs and 1 g para-aminothiophenol were dispersed in 20 mL of DMF solvent by sonication, followed by

microwave irradiation at 130 °C for 10 min. Fourier transform infrared spectroscopy (FTIR) was used to characterize all of the SWCNT s intermediate products.

Synthesis of Gold Nanoparticles (GNPs) and binding with functionalized SWCNTs

All of the glassware used in the following procedures was cleaned in a bath of freshly prepared 3:1 HCl: HNO_3 and rinsed thoroughly with nanopure H_2O prior to use. According to our slighty modified Frens[1,2] method, the gold nanoparticles (GNPs) were prepared by heating a 50 mL solution of 0.01% $HAuCl_4$ to its boiling point and adding 0.6 mL of 1% sodium citrate. The mixture was then boiled for an additional 10 min, and the mixture was allowed to cool. The gold nanoparticle solution can be stored at 4 °C for one month. JEM-2100F transmission electron microscope (TEM) and Ultraviolet–visible spectroscopy (UV-Vis) were used to characterize the shape and size of the gold nanoparticles.

For binding the gold nanopartilces to the functionalized SWCNTs, 10 mg of SWCNTs were added to 10 mL H_2O nanopure H_2O and dispersed via sonication for 10 min. A 0.5 mL solution of gold nanoparticles was then added to the SWCNT mixture dropwise, and the mixture was agitated until the dark red color remained for over 15 min. The mixture was then centrifuged to remove the extra unbound gold nanoparticles, and nanopure H_2O was added to make the final volume of the SWCNTs-GNPs mixture to be 10 mL. TEM and UV-Vis were used to characterize and visualize the gold nanoparticles decorated SWCNTs.

Preparation of monoclonal antibody (MAB) conjugated SWCNTs-GNPs

For selective sensing of MDR *Salmonella typhimurium DT104*, we modified the GNP surface with monoclonal antibody AC04. For this purpose, first, 80μL of 10^{-4} M thiol ending polyethylene glycol (HOOC-PEG-SH) was added into 1 mL SWCNTs-GNPs mixture and stirred for 20 min at room temperature. This mixture was allowed to react at 4 °C overnight for the -SH group to form a bond with the GNPs while leaving a free carboxyl group at the other end. Excess HOOC-PEG-SH was removed by centrifugation at 4000 rpm for 1 h at room temperature (3 times). Phosphate-buffered saline (PBS) was then added to make the final volume 1 mL.

In next step, covalent immobilization of the amine group of the antibody onto the carboxyl groups of PEG-SH was performed by ethyl-(dimethylaminopropyl)-carbodiimide (EDC) / N-hydroxysuccinimide (NHS) coupling catalysis. For this purpose, 15 μL of EDC (1 mg/mL) and 15 μL of Sulfo-NHS (1 mg/mL) were first added into 1 mL of SWCNTs-GNPs modified with

PEG-SH solution, which will active the carboxyl group in the terminus of PEG to bind with antibody. Then 15 μL (10 μg/mL) of antibody was added to the aforementioned solution. The solution was stirred for 2 min, and the reaction was allowed to proceed for 2 h at room temperature, and stored for 12 h at 4 °C. The final SWCNTs-GNPs-antibody suspension was centrifuged at 2,000 rpm for 10 min to remove any unbound antibodies. The residue was redissolved in 1 mL of PBS solution and was stored at 4 °C for use. This process was repeated for 3 times. The solution could be stored at 4 °C for at least 1 month.

Bacteria Culture and incubation with Antibody-Modified SWCNTs-GNPs

Salmonella typhimurium DT104, which is resistant to five different antibiotics such as ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline, was purchased from ATCC (ATCC 700408). The MDR bacteria were cultured by following the ATCC protocol as instructed. Initially, the supplied pellet of *DT104* was rehydrated on 5 to 6 mL Bacto™ Tryptic Soy Broth (TSB) and incubated at 37^{o}C for 24 h. Next, from the growth culture, a loop of bacteria were streaked on tryptic agar plate and incubated for 24 h at 37^{o}C. A tryptic agar plate was made with Difco^{TM} tryptic soy agar (TSA). A single colony from the TSA plate was inoculated into 10 mL TSB and incubated at 37 ^{o}C for 16 h in a shaker at 150 rpm, which resulted in an inoculum of 10^{8} CFU/mL. All of the growth medium and Agar were autoclaved at 121^{o}C for 15 min at high pressure (0.1 MPa) before the experiment. M3038-modified SWCNTs-GNPs (10μL) were added to 100μL of solution containing 10–10^{7} CFU/mL of *Salmonella DT104*, suspended in 1×PBS with 0.5% BSA. The mixtures were incubated at room temperature for 20 min and were then washed 3 times with 1×PBS buffer.

Surface Enhanced Raman Spectroscopy (SERS) Probe for Targeted Sensing

For the SERS experiment, we used a continuous wavelength diode-pumped solid-state laser (DPSS) laser operating at 670 nm as an excitation light source. For excitation and data collection, we used InPhotonics 670 nm Raman fiber optic probe, which is a combination of 90 mm excitation fiber and 200 mm collection fiber. For Raman signal collection, we used a miniaturized QE65000 Scientific-grade Spectrometer from Ocean Optics, with a response range of 220–3600 cm^{-1}. The Hamamatsu FFT-CCD detector used in the QE65000 provides 90% quantum efficiency with high signal-to-noise and rapid signal processing speed as well as remarkable sensitivity for low-light level applications. The Raman spectrum was analyzed with Ocean Optics data acquisition SpectraSuite spectroscopy software.

Photo thermal exposure and determination of live bacteria percentages

For photo thermal destruction experiments, we applied a continuous wavelength laser (operating at 670 nm, with a power of 1-2 W cm^{-2} as an excitation light source) to our hybrids for 10–20 min. Then, the bacteria were transferred to tryptic agar plate after photo thermal destruction, which was incubated for 24 h at 37^{o}C. The colony number for each countable plate was then counted with a colony counter. For accuracy, each experiment was performed 3 times.

References:

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2. G. Frens, *Nature Phys. Sci.*, 1973, **241**, 20.