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A facile method for synthesis of gold nanotubes and their toxicity assessment

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ABSTRACT

Gold nanotubes hold great potential for various biomedical applications such as gene transfer, tissue engineering, image processing, biosensors, in nanopore sequencing and hence they need to be non-toxic and biocompatible. In this context, we present a partially green approach for gold nanotube synthesis and determine their toxicity using *in vitro* assays. The gold nanotubes were synthesized using sodium sulphate nanowires as sacrificial template on which gold nanoparticles produced in *Escherichia coli DH5α* were deposited to get a tubular structure. The gold nanoparticles, sodium sulphate nanowires and gold nanotubes were characterized using transmission electron microscopy. The toxicity of gold nanotubes was determined using Trypan blue assay and lactate dehydrogenase (LDH) assay. The cytotoxicity profile of these nanotubes was also compared with the cytotoxicity profile of nanotubes synthesized by complete chemical approach. Results showed that the use of biogenic gold nanoparticles in preparing the nanotubes has significantly decreased the cytotoxicity as compared to nanotubes prepared using chemical method. The % cytotoxicity with trypan blue assay and LDH assay ranges from 8.2 to 33% and 8.8 to 31.2%, respectively with increasing concentration of gold nanotubes (0.1–10 mg/ml) for biogenic nanotubes.

1. Introduction

Nanotubes are found to be at the leading edge of the rapidly developing field of nanotechnology because of their diverse applications in diagnostics, *in vitro* and *in vivo* imaging, biosensing, drug delivery and thermal ablation techniques, and immunoassay [1–5]. Nanotubes offer distinct outer & inner surfaces for attachment of molecules like antibodies, receptors, aptamers on its surface or encapsulation of drugs for therapeutics. Carbon nanotubes have been extensively studied for their biomedical applications but have toxicity related issues [6]. Alternatives of carbon nanotubes are explored to overcome the toxicity related issues. Gold nanostructures such as nanoparticles, nanorods, nanocages, nanoshells are popularly used in diagnosis and therapeutics of various disorders and have lesser biocompatibility issues [7–9]. They are popularly used for *in vivo* bioimaging of tissues as they preferentially absorb light in near-infra red regions with low background signal [10]. Further, these structures show significant photothermal effects which is being used for photothermal ablation or therapeutics [11,12]. Since nanotubes have distinct advantage of offering two surfaces, they have the potential to be used in targeted therapy and in theranostics *i.e.* same molecule can be used for both imaging and therapeutic of disease. The potential of gold nanotubes (AuNTs) in biomedical applications have not been explored yet.

Synthesis, control of size, optical properties, cytotoxicity, biodistribution, and clearance are various important aspects to be considered while engineering a nanostructure. First and foremost is the facile, non-toxic and safe method for synthesis of biocompatible nanotubes. Most of the methods described for synthesis of gold nanotubes are template based which utilized prefabricated nanostructures like organic–inorganic substrate, porous membrane, metal nanorods and nanowires, carbon nanotubes as a sacrificial template [13–18]. Recently, controlled length synthesis of gold nanotubes have been demonstrated using a chemical method but these nanotubes were made biocompatible by surface coating of poly(sodium 4-styrenesulfonate) [19]. Simple and safe methods for fabrication of non-toxic gold nanotubes shall be explored.

In the present paper, a template based method has been used for synthesis of gold nanotubes. Herein, gold nanoparticles synthesized using green chemistry approach (*Escherichia coli*

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DH5α) and chemical method (sodium borohydride reduction), were separately deposited on a sacrificial sodium sulphate nanowire template. The removal of water dissolvable template leads to the synthesis of gold nanotubes. The paper further assesses the safety of two types of synthesized nanotubes on white blood cells using *in vitro* methods *viz*; trypan blue and LDH assay and a comparison was drawn between the two.

2. Materials and methods

All reagents and chemicals used were of analytical grade or HPLC grade. Silver nitrate ($AgNO_3$), Sodium dodecyl sulfate (SDS), Triton X-100 were purchased from Merck. Cetyl trimethyl ammonium bromide (CTAB), Dimethyl formamide (DMF) was purchased from Sigma–Aldrich. Lactate dehydrogenase (LDH) assay kit was purchased from Cytoscan. Stannous chloride ($SnCl_2{\cdot}2H_2O$), isopropanol was purchased from CDH. Trypan blue dye was purchased from Amresco. De-ionized water was prepared on a Milli-Q laboratory plant. The blood was collected freshly from a healthy human donor in EDTA vacutainer. The Gram-negative, rod shaped bacteria *E. coli DH5α* were used as bio-nanofactory to bioreduce the aurochloric acid ($HAuCl_4$) and modulate the formation of gold nanoparticles.

2.1. Synthesis of gold nanotubes

AuNTs were synthesized by depositing AuNPs on the sodium sulphate nanowire template. Subsequent dissolution of nanowires leads to a tubular structure. In the paper, AuNPs were synthesized using *E. coli DH5α* as well as by sodium borohydride reduction and then deposited on the template for nanotube formation.

2.1.1. Synthesis of gold nanoparticles using E. coli DH5α

500 ml of bacterial culture was grown in LB broth by adding overnight grown seed culture under shaking conditions for 6–7 h at 37 °C. The bacterial cells in log phase were harvested by centrifuging the broth at 4000 rpm for 5 min and resuspended in 30 ml of sterile distilled water after thorough washing. An aqueous solution of $HAuCl_4$ was added to the cell suspension, so that the final concentration of gold ions in the reaction mixture is 1 mM. The mixture was incubated at 37 °C under shaking conditions of 120 rpm for 120 h. With the addition of $HAuCl_4$, the color of the solution turned light yellow and finally dark purple with subsequent incubation. After centrifugation, the supernatant was analyzed with UV–vis spectroscopy and absence of peak indicated that the nanoparticles were not present in this fraction. To release the nanoparticles from the bacterial cells, cell pellet was treated with the 1% Triton-X100 with intermittent shaking. The cell debris was removed by centrifuging at 3500 rpm, 10 min and supernatant was used for characterization of Au-NPs.

2.1.2. Sodium borohydride synthesis of gold nanoparticles

The Synthesis of AuNPs was done in toluene following the protocol of Brust et al [20]. with slight modification. An aqueous $HAuCl_4$ (29.1 mM) solution was taken and mixed with toluene containing TOAB (49.6 mM) and shook for 5 min till two phases were clearly visible. Aqueous phase was removed and darker solution containing $HAuCl_4$ was added to $NaBH_4$ (353 mM) drop wise within one minute. Generation of dark red color indicated formation of nanoparticles. Solution was left for at least one hour with vigorous stirring. After one hour of vigorous stirring, impurities present in the form of water were removed by adding HCl and NaOH, respectively, this solution was kept overnight with vigorous stirring and stored at 4 °C.

2.1.3. Synthesis of water-soluble sodium sulfate nanowires

The synthesis of water soluble Na_2SO_4 nanowires was done by following the protocol of Pu et al [21]. with slight modification. Sodium sulphate nanowires were synthesized by refluxing 0.02 M sodium dodecyl sulphate (SDS), 1 mM silver nitrate ($AgNO_3$), 4 mM $SnCl_2{\cdot}2H_2O$, 2 mM cetyltrimethyl ammonium bromide (CTAB) in 5 ml DMF at 160 °C in silicon oil bath for 60 min under stirring until the solution turns to a milky white color. The solution was removed from the hot plate and cooled to room temperature. The white precipitate was separated by centrifuging at 10,000 rpm for 5 min and the precipitate was washed with 2-propanol. The obtained precipitate was stored in 2-propanol until the further use.

2.1.4. Synthesis of gold nanotubes

Metallic nanoparticle nanotubes have been synthesized by depositing metal nanoparticles in the porous walls of template [21]. Herein, the AuNPs from *E.coli* and borohydride reduction were deposited on sodium sulphate template to obtain tubular structures. In brief, 100 μl of AuNPs solution was directly added to 1 ml of Na_2SO_4 nanowires solution. The mixture was incubated for 10 min after sonication for few seconds. The gold nanotubes were then collected by centrifuging at 13,000 rpm for 10 min and then washed several times in autoclaved distilled water.

2.2. Characterization of gold nanoparticles, sodium sulfate nanowires and gold Nanotubes

Gold nanoparticles synthesized using two routes were analysed using UV–vis spectroscopy, dynamic light scattering (DLS) and transmission electron microscopy (TEM). Sodium sulfate nanowires and gold nanotubes were analysed using TEM carried out on JEOL 2100F transmission electron microscopy operating at 200 kV. Samples for TEM characterization were prepared by placing a drop of the solution onto carbon coated copper grid and dried at room temperature.

2.3. Toxicity assessment of gold nanotubes

The toxicity assessment of AuNTs synthesized using *E.coli* AuNPs and borohydride reduced AuNPs was done using trypan blue assay and lactate dehydrogenase assay on human white blood cells (WBCs). The toxicity of synthesized AuNTs was determined by treating their different concentrations (10 mg/ml, 5 mg/ml, 1 mg/ml, 0.5 mg/ml, 0.25 mg/ml, 0.1 mg/ml) with human white blood cells for 1 h in respective assays. The statistical calculations were done by computing mean ± Standard deviations in set of five replicate assays for each test.

WBCs were isolated from healthy human blood with their consent and used following all safety measures. 3 ml of fresh blood was collected in EDTA vacutainer. To this, 10 ml of Red cell lysis buffer (0.84% of Ammonium chloride solution) was added in a 15 ml falcon tube. After incubation for 20 min at 37 °C, WBCs were collected by centrifuging the blood at 2000 rpm for 10 mins. The supernatant was discarded and pellet was washed twice with PBS or RPMI medium by centrifuging at 1500 rpm for 5–6 min. The obtained pellet was resuspended in RPMI medium for further use. The cell counting was done by using Neubauer chamber.

2.3.1. Trypan blue exclusion assay

The viability of cells was checked by using the trypan blue dye which is taken up by the dead cells and excluded by the live cells. The assay was carefully repeated multiple times by the same researcher to minimize manual errors. After incubating WBC's with AuNTs, cells were washed and mixed with Trypan blue in the 1:1 ratio and observed under microscope for the viability.