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SYNTHESIS OF THIN WALLED SILICA-COATED SILICON NANOTUBES AND THEIR ANTIBACTERIAL STUDY

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Abstract

Silica-coated silicon nanotubes (SNTs) were synthesized using direct arc plasma assisted gas phase condensation. Transmission electron microscopy showed that SNTs exhibited average diameter of 14 nm with length of several 100 nm. The wall thickness was found to be less than 1 nm which is the first report of its own kind. The anti bacterial activity of these nanotubes was studied for two Gram-positive and Gram-negative bacteria using the optical densitometric technique, and by determining colony-forming units. SNTs showed good activity against all the tested bacteria. Specifically SNTs were found to inhibit multidrug-resistant S*taphylococcus aureus* at 10 µg/ml (IC 50 = 100 µg/ml).

Keywords

Silicon nanotubes, Thermal plasma synthesis, Thin-walled, Antibacterial activity.

Introduction

Nanotubes have a significant impact owing to larger surface reactivity, compared to other nanostructures. Since the discovery of carbon nanotubes [1] (CNTs), there has been a considerable interest in silicon nanotubes (SiNTs) in view of the technological hierarchy of silicon in various fields. Electronic and structural properties of hypothetical SiNTs, similar to those of CNTs, were compared. Stereo chemically dependent band structures and conducting properties were being calculated [2]. It is difficult to synthesize carbon like silicon nanotubes due to preferred sp^3 hybridization by silicon rather than sp^2 as in case of carbon [2]. However, high pressure and high temperature synthesis conditions are conducive for the formation of this metastable state.

There are few reports of synthesis of silicon nanotubes using pulsed laser ablation [3], chemical vapor deposition [4-5], the template method [6]. Tang *et al.* [7] synthesized silicon nanotubes with wall thickness of nearly 5 nm using supercritical hydrothermal conditions. Most of these methods involve the use of harmful silicon derivatives and nanotubes synthesized by most of these methods have a wall thickness of the order of several nanometers. We present our work on the synthesis of silica-coated thin-walled silicon nano-tubes (SNTs), using thermal plasma-assisted gas phase condensation technique a wall thickness of less than one nanometer. Morphology of silicon nanotubes was studied by transmission electron microscopy. Elemental composition was investigated using energy dispersive X-ray spectroscopy and chemical composition and structure was analyzed using Raman spectroscopy.

These nanotubes were then subjected to antibacterial study, as it is important for its application in antibacterial coating applications in medical field. The antibacterial activity of as synthesized nano-structures was studied for selected strains of bacteria that are pathogenic and frequently colonize medical devices. Two Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and two Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria were used for the study. Minimum inhibitory concentration (MIC) was determined using optical densitometric technique and by measuring colony forming units.

Method

SNTs were synthesized in a DC arc thermal plasma reactor. The plasma reactor consisted of a graphite anode (in the shape of a crucible) and a tungsten cathode (in the shape of a 4 mm diameter rod) mounted in a vertical geometry, housed inside a double walled water cooled stainless steel chamber. The silicon powder from CDH, India Ltd. was placed on the graphite anode. Before exciting the arc the chamber was evacuated to a base pressure of about 10^{-3} mbar. It was filled with the

mixture of argon and hydrogen (molar ratio 95:5) gas so as to maintain a pressure of 700 mbar. Arc was then struck between the anode and the cathode at an arc voltage of 12 V ± 0.4 V and an arc current of 80 A ± 5 A. Silicon gets vaporized due to the arc and results in the formation of nanoparticles via homogeneous nucleation. The nanoparticles are collected from the walls of the chamber. The samples were characterized by Transmission Electron Microscopy (TEM) (Technai G^2 20 microscope with LaB_6 filament and Charge Coupled Device (CCD) Camera), Scanning Electron Microscopy (SEM) (JEOL JSM-6360A) with attached Energy Dispersive X-ray spectroscopic (EDX) unit, Raman spectroscopy with Jobin Yvon Labram HR800 spectrometer.

The Gram positive bacterial strains like *Staphylococcus aureus* (National collection of Industrial Microorganism (NCIM) 2079), *Bacillus subtilis* (NCIM 2063) and Gram negative strains like *Escherichia coli* (NCIM 2065) and *Pseudomonas aeruginosa* (NCIM 2200) were procured from NCIM, India. The above bacterial strains were cultured on to Nutrient Broth Media (10 g Yeast extract, 5g sodium chloride, 10g tryptone, 20g Agar in one litre media and pH maintained between 7-7.5). All the bacteria were grown at 37°C overnight. The population of microbes was maintained between 1 x 10^8 to 5 x 10^9 CFU/ml except *B. subtilis*.

MIC of the sample was estimated using the micro dilution method by determining the optical density and colony forming units. The experimental miniprep was prepared having following components: 900µl of NB media, 100µl of respective bacterial inoculums, SNTs in varying concentrations (0, 10, 50, 100, 150 & 200 µg/ml) and sterile distilled water was added to equate the reaction volume. The strains were incubated at 37°C over night. Each and every test concentrations along with control was diluted upto 10^9 dilutions using 0.9% sodium chloride.

To determine the MIC values using the optical densitometric technique, 100 µl of all tested concentrations of inoculums, their

dilutions up to 10^9 were added to 96 well plates and absorbance at 600nm was taken. NB media and Saline were used as negative control. Experimental mixture without SNT (100 µl) was used as positive control. From the above experimental miniprep, 100 µl of sample was used from each and every test concentrations having dilutions of 10^9, 10^8, & 10^7 to plate the bacterial inoculums including control. In *B. subtilis,* plating was carried out in dilutions of 10^3, 10^4 & 10^5 owing to low optical densitometric measurements. The colony forming units were determined by counting the bacterial colonies and then by multiplying with the dilution factor. All assays were carried out in duplicate.

Results and Discussion

Fig. 1 shows the TEM micro-graph of SNTs. It shows that the sample contains nanotubes as well as nanoparticles. The nanotubes and nanoparticles are found to be in the ratio of 70:30. The nanoparticles have size variation between 5-25 nm while the diameter of the nanotubes varies between 9 nm and 30 nm. Maximum tubes have diameter around 14 nm while the lengths of nanotubes were of the order of several 100 nm.

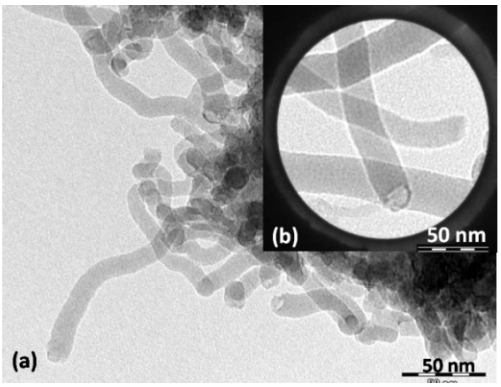

Fig 1.a. TEM micrograph of silica-coated silicon nano-tubes. b. Tip of single nanotube.

Tubular formation is clearly evident from the circular open tip of a single nanotube, shown in the inset (Fig 1.b). The thickness of the

annular dark wall seen at the tip happens to be less than 1 nm.

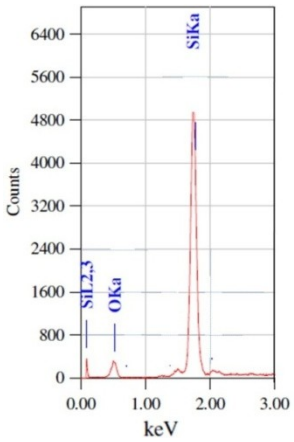

Fig 2. SEM-EDX spectra for silica-coated silicon nano-tubes

Fig. 2 shows the SEM-EDX spectrum for SNT. The spectrum shows the presence of peaks at 0.525, 0.129 and 1.739 keV which corresponds to O K_α, Si $L_{2,3}$ and Si K_α edge respectively. It is observed that the ratio of silicon to oxygen for SNT is 1.27. This indicates that the tubes are not fully oxidized.

Fig. 3 shows the Raman spectrum for SNT. The peak for crystalline silicon is symmetric and appears at 520.5 cm^{-1} with full width half maximum (FWHM) of the peak to be 2.8 cm^{-1}. Raman spectra for SNT show an asymmetric peak at 511.5 cm^{-1} with an FWHM of 22 cm^{-1}. The peak for SNT is red shifted by 9 cm^{-1}.

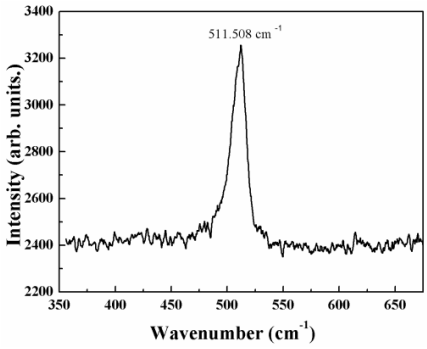

Fig 3. Raman Spectra of silica-coated silicon nano-tubes

It was found that the concentration of nanotubes at which the growth was inhibited was different for different bacteria. SNTs showed MIC of 10µg/ml for *S. aureus.* IC-50 is found to be 100µg/ml. The center for

disease control and prevention, reports that the number of annual multidrug-resistant *Staphylococcus aureus* (MRSA) infections increased from 127,000 to 278,000 between 1999 and 2005 [7]. In this scenario, SNTs are found to be potential candidate to target MRSA infections. Inhibition of *E.coli* and *P. aureginosa* by SNTs is found to be at 10µg/ml. The growth of *B. subtilis* cultures were not seen inhibited. Minimum Inhibitory Concentration (MIC) was found to be of the order of microgram for SNTs, which was comparable and in many cases less than those reported in metal oxides for both gram positive as well as gram negative bacteria [7].

In order to measure the viable cells, colony forming units (CFU units) were determined using serial dilutions of suspensions followed by spread plate colony counting. The *B. subtilis* cultures exposed to SNTs showed definite pattern of reduced viability (Fig. 5) with increase in SNTs concentration. The approximate IC-50 value of SNTs is 200µg/ml in *B. subtilis* cultures. The 100µg/ml proved to be effective in controlling the *S. aureus* even at very low concentration with IC-50 of 10µg/ml. *For E. coli,* MIC was found out be 10µg/ml for SNTs, which is in concurrence with densitometric analysis. In *P. aeruginosa,* 50µg/ml was found to be effective in reducing the viability.

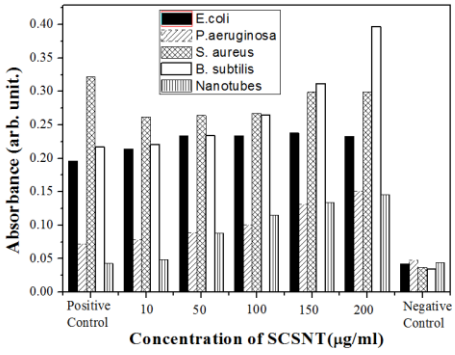

Fig 4. Effect of different concentrations of SNT on bacterial strains tested. Standard absorbance values of SNT at various concentrations from 0 g/ml (+ve control) to 200 lg/ml are provided. NB media alone was used as negative control.

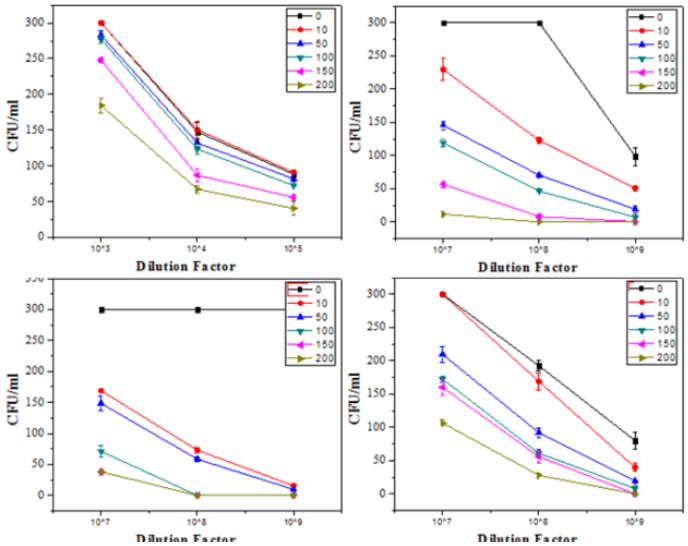

Fig 5. CFU counting in Grampositive bacterial strains calculated for different concentrations of SNT (0–200 lg/ml). a. CFU of B. subtilis b. CFU of E. coli c. CFU of S. aureus d. CFU of P. aeruginosa.

Conclusion

We have shown that the growth of thin walled nano-tubes (< 1 nm) is possible by thermal plasma assisted gas phase condensation, which is a clean method of synthesis. We have shown that silica coated silicon nano-structures can be a good substitute as a cheaper and biocompatible antimicrobial agent. The low values of MIC are encouraging and are comparable to those reported to other oxide nanomaterials [7].

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