Fabrication of bismuth subcarbonate nanotube arrays from bismuth citrate†

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Bismuth subcarbonate ($(BiO)_2CO_3$) nanotubes with uniform diameters of about 3 ~ 5 nm were fabricated from bismuth citrate; these nanotubes exhibit antibacterial properties against *Helicobacter pylori* (50% inhibition at 10 μg mL^{-1}), a bacterium causing peptic ulcers and gastritis.

Synthesis of inorganic nanotubes has attracted considerable attention since the discovery of carbon nanotube by S. Iijima,[1] due to their fundamental significance and potential applications.[2] The general synthetic strategies involve arc discharge, laser ablations, hydrothermal process, and surfactant-assisted synthesis.[3] Various attempts have been made to synthesize different nanotubes under mild conditions.[4–8] The synthesis of metallic bismuth and antimony nanotubes using a low-temperature hydrothermal reduction method has been reported.[9] Bi_2S_3, β-Bi_2O_3, Bi_2Te_3 and Bi_2Se_3 nanotubes were also prepared through rational synthesis route.[10]

There has recently been an increasing interest in the use of nanoparticles for medical and healthcare purposes. Silver based nanoparticles have been found to be an effective antimicrobial agent against *E. coli*, *S. aureus* and *Ps. Aeruginosa*, and anti-HIV agent.[11] Metal oxide based nanoparticles such as magnesium oxide (MgO) and titanium dioxide (TiO_2) also exhibit antibacterial activity.[12,13]

Together with antibiotics, bismuth compounds such as colloidal bismuth subcitrate (De-Nol®), ranitidine bismuth citrate (Pylorid®), bismuth subsalicylate (Pepto-Bismol®) and bismuth subcarbonate ($(BiO)_2CO_3$, BSC), have long been used in clinics for the treatment of *Helicobacter pylori* (*H. pylori*) infection, peptic ulcers and other gastrointestinal disorders.[14]

In this communication, we report a large-scale preparation of bismuth subcarbonate ($(BiO)_2CO_3$) nanotubes by a simple reflux of bismuth citrate and urea in ethylene glycol.‡ Bismuth citrate was used as a precursor for both bismuth and carbonate. More importantly, the polymeric structure of bismuth citrate serves as a template for the synthesis of nanotubes. To our knowledge, this is the first report on the fabrication of bismuth subcarbonate nanotubes.

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The XRD spectrum of the synthesized complexes showed that almost all the diffraction peaks can be readily indexed to the tetragonal $(BiO)_2CO_3$ (JCPDS no. 41-1488), supporting the fact that the sample is bismuth subcarbonate ($(BiO)_2CO_3$) (Fig. S1, ESI†). X-Ray photoelectron spectroscopy (XPS) was used for evaluating its composition and purity. In the XPS and high-resolution XPS spectra, two strong peaks in the Bi region at 158.8 and 164.1 eV are assigned to the Bi(4f) binding energy. The peaks in the carbon region at 284.2 and 288.8 eV and oxygen region at 530.1 eV correspond to the C(1s) and O(1s) transition, respectively. The ratio of the peak integrations was found to be 2 : 1 : 5 for Bi, C and O, respectively, indicative of an atomic ratio of Bi : C : O = 2 : 1 : 5, *i.e.* the formulation of $(BiO)_2CO_3$ (Fig, S2, ESI†). The nanostructure of these nanotubes was also investigated by transmission electron microscopy (TEM), scanning electron microscopy (SEM, Fig. S3, ESI†) and selected-area electron diffraction (SAED). The TEM images reveal that large amount of $(BiO)_2CO_3$ nanotubes and nanotube arrays were prepared (Fig. 1).

Their diameters are in a narrow range from about 3.2 to 5.2 nm, with an average diameter of 4.0 ± 0.5 nm (Fig. S4 ESI†). The length of the nanotubes varies from several hundred nanometers to several micrometers. The aspect ratio, which is the ratio of length

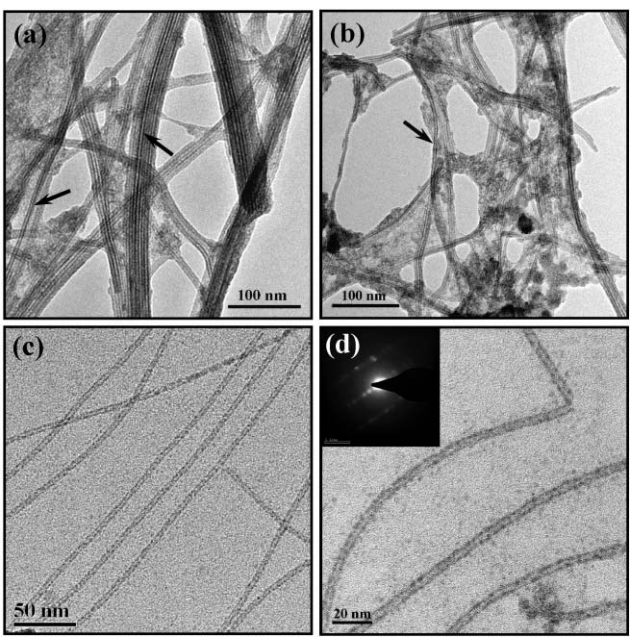

Fig. 1 Typical TEM images of the $(BiO)_2CO_3$ nanotubes obtained in the absence ((a) and (b)) and in the presence ((c) and (d)) of CTAB. Note that the insert of (d) corresponds to the SAED pattern of a nanotube.

to diameter of the nanotubes, varies from 100 to 200. Most of the nanotubes are aligned together to form bundles of nanotubes although individual $(BiO)_2CO_3$ nanotubes are visualized as indicated with the arrow line, Fig. 1a and 1b. In contrast, well separated individual $(BiO)_2CO_3$ nanotubes were observed in the presence of the surfactant, cetyltrimethylammonium bromide (CTAB) (Fig. 1c and 1d, *vide infra*).

The high resolution TEM (HRTEM) images of well-crystallized $(BiO)_2CO_3$ nanotube arrays are shown in Fig. 2. As depicted in Fig. 2a, two $(BiO)_2CO_3$ nanotubes are lying side by side with nearly identical diameters (4.2 and 4.3 nm). The fringe spacing is *ca.* 0.278 nm, which is very close to the interplanar spacing of the (110) lattice planes (2.734 Å) of $(BiO)_2CO_3$ (Fig. 2b). The SAED pattern, corresponding to the bundle of $(BiO)_2CO_3$ nanotubes (Fig. 2a, insert) showed orderly arranged spots, which is a characteristic diffraction pattern for tubular nanomaterials and could be indexed to (110), indicative of a high order of crystallinity morphology of $(BiO)_2CO_3$ nanotubes. This is in good agreement with the well-crystallized structure deduced from HRTEM and these nanotubes may therefore have preferential [110] growth along the axial direction.

Probably due to the low melting point, the morphology of some individual nanotubes has become blurry after long time electron beam irradiation during the TEM examination. Similar behavior was noticed in other reports on the characterization of nanotubes.[9] A single nanotube was observed to melt to form $(BiO)_2CO_3$ particle line upon long-time beam irradiation (Fig. S5, ESI†). In spite of this, these $(BiO)_2CO_3$ nanotubes were relatively stable compared to metallic bismuth nanotubes, being highly sensitive to the electron beam irradiation due to their low melting point.[9]

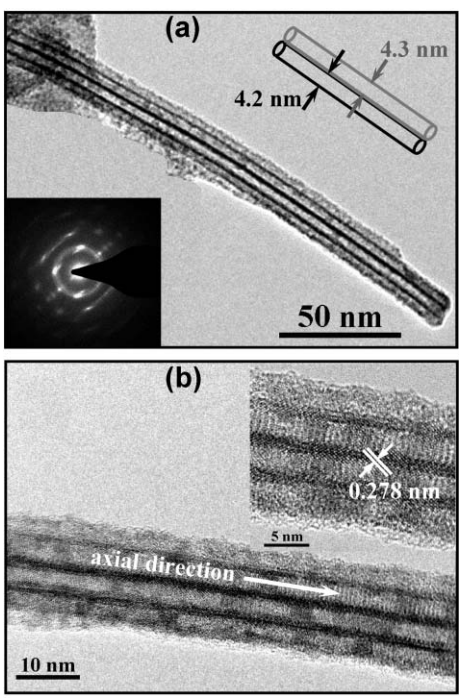

Fig. 2 High-resolution TEM images of a bundle of $(BiO)_2CO_3$ nanotubes and its corresponding SAED pattern (insert 2a). Note that the *d*-spacing between the lattice planes (0.278 nm) corresponds to the (110) plane.

Energy dispersive X-ray analysis (EDX) was used to analyze the elemental compositions of these nanotubes lying on the hole of the copper grid, where the carbon coating was absent in this area (Fig. S6, ESI†). The presence of bismuth, carbon and oxygen peaks clearly confirmed that these nanotubes are $(BiO)_2CO_3$, which is in agreement with XRD data. The presence of a copper peak is due to the copper grid.

To further confirm the presence of bound-carbonate in these nanotubes, a solid-state ^{13}C MAS NMR of the $(BiO)_2CO_3$ nanotubes was performed. Only a peak at 168 ppm was observed in the solid-state ^{13}C NMR spectrum of $(BiO)_2CO_3$ nanotubes (Fig. S7, ESI†). The chemical shift is similar to the bismuth bound carbonate (CO_3^{2-}) in the carbonate–Bi^{3+}–transferrin ternary complex (the metal bound CO_3^{2-} at *ca.* 166 ppm),[15] again indicating that the nanotubes are $(BiO)_2CO_3$.

The mechanism of formation of $(BiO)_2CO_3$ nanotubes is not well understood. Bismuth citrate may play an important role in the fabrication of $(BiO)_2CO_3$ nanotubes. It may serve as a template to direct the formation of 1-D linear $(BiO)_2CO_3$ nanotubes due to the linear polymeric structure of bismuth citrate ($[Bi(citrate)_2Bi)]_n^{2n-}$).[16] Previously, controlled synthesis of Bi_2S_3 nanorods were made using bismuth citrate as a template.[16b] Indeed, no 1-D tube or linear rod-like nanomaterials was observed when bismuth nitrate pentahydrate was used under identical conditions (data not shown). Thus, bismuth citrate may only disperse in the solvent and retain its linear polymeric structure, which could serve as a template to assist the growth of 1-D nanostructure. Previously it was found that CTAB, an excellent capping reagent, could be used to prepare stable and well-dispersed nanoparticles or nanorods.[16b,17] Here, we found that well-separated $(BiO)_2CO_3$ nanotubes were obtained in the presence of an appropriate amount of CTAB (Fig. 1c), indicating that CTAB stabilizes these nanotubes as illustrated in Fig S8, ESI.†

To investigate whether these nanotubes remain antimicrobial activity, their inhibitory activities against *H. pylori* were evaluated by a standard method.§ The minimum inhibitory concentration (MIC) was determined by the measurement of the OD values at 600 nm after 3 d of incubation. The inhibition was found to be bismuth concentration dependent: >80% inhibition at 80 μg mL^{-1} of $(BiO)_2CO_3$ nanotubes; 68% at 20 μg mL^{-1} and 50% at 10 μg mL^{-1} (Fig. 3). The MIC_{50} value was evaluated to be 10 μg mL^{-1}. The anti-*H. pylori* activities of colloidal bismuth subcitrate and bismuth oxide nanoparticles were also examined under identical conditions for comparison. As shown in Fig. 3,

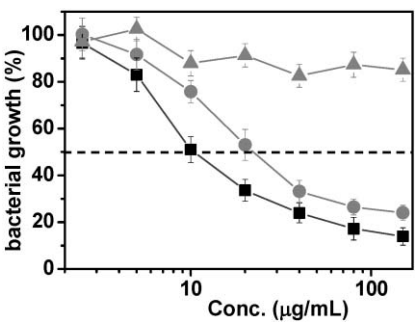

Fig. 3 Inhibition profiles of $(BiO)_2CO_3$ nanotubes (■) together with colloidal bismuth subcitrate (●) and Bi_2O_3 nanoparticles (▲), toward *H. pylori*.

slightly enhanced inhibitory properties were found for the $(BiO)_2CO_3$ nanotubes compared with the clinically used antiulcer drug, colloidal bismuth subcitrate (CBS), and almost no inhibitory properties were found for bismuth oxide nanoparticles.

The synthesis of $(BiO)_2CO_3$ nanotubes under moderate conditions could be useful for its potential medical applications. Recently, novel bismuth triple and quadruple therapeutic approaches for *H. pylori* infection have been recommended.[14b,18] Based on this, a single bismuth-based triple therapy monocapsule containing colloidal bismuth subcitrate, tetracycline and metronidazole was developed. This drug is not a simple mechanical mixture but a smaller capsule inside a bigger capsule (a bismuth compound), to prevent possible reactions among these drugs. $(BiO)_2CO_3$ nanotube may be a good candidate as a "capsule" due to its unique nanostructure (*e.g.* size), antimicrobial activity and digestible feature under acidic conditions. Moreover, these nanotubes can also be used as carriers for other drugs.

In summary, the fabrication of $(BiO)_2CO_3$ nanotubes under moderate condition has been reported and the nanotubes have been characterized. Importantly, these nanotubes showed antibacterial properties against *H. pylori*, a bacterium causing peptic ulcers and gastritis. Ongoing study is to fill other drugs into the nanotubes to form "nanodrugs" for treating *H. pylori* infection and possibly other diseases.

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Notes and references

‡ **Synthesis**: In a typical procedure, 0.3 g (0.75 mmol) bismuth citrate and 0.135 g (2.25 mmol) urea were dissolved into 50 ml ethylene glycol in a round-bottom flask. The mixture was stirred and sonicated until all the chemicals were dissolved. The mixture was refluxed at 200 °C for 1 h under oil bath with continuous vigorous stirring. After cooling back to room temperature, the mixture was centrifuged to collect the black solid product. The solid product was first washed twice with both acetone and water followed by centrifugation. Finally, the solid product was dried in a desiccator at room temperature for further characterization. The sample in the presence of CTAB (0.1 mmol) was obtained under similar conditions. § **Biological studies and measurement**: *H. pylori* 26695 (a gift from Li Ka Shing Faculty of Medicine, the University of Hong Kong) was cultured on Brucella agar (Difco) plates supplemented with 10% defibrinated sheep blood at 37 °C under microaerophilic conditions (5% CO_2, 4% O_2 and 91% N_2), maintained by Campypak Plus (BBL). A series of bismuth compounds with different concentrations were added to each well which contains the same amount of *H. pylori* suspensions and cultured at 37 °C under microaerophilic conditions (5% CO_2, 4% O_2 and 91% N_2) for 3–4 days. Together with the control, the optical density at 600 nm (OD_{600}) of each well was measured and the bacterial survival percentages were determined.

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