Antibacterial Activities of Tellurium Nanomaterials

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Abstract: We prepared four differently shaped Te nanomaterials (NMs) as antibacterial reagents against Escherichia coli. By controlling the concentrations of hydrazine (N₂H₄) as reducing agent, NaCl, and temperature, we prepared Te nanowires, nanopencils, nanorices, and nanocubes. These four Te NMs resulted in a live/dead ratio of E. coli cells of less than 0.1, which is smaller than that of Ag nanoparticles. The order of antibacterial activity against E. coli is nanocubes ≈ nanorices > nanopencils ≈ nanowires. This is in good agreement with the concentration order of tellurite (TeO₃²⁻) ions released from Te NMs in E. coli cells, revealing that TeO₃²⁻ ions account for the antibacterial activity of the four Te NMs.

Keywords: antibiotics · nanomaterials · semiconductors · tellurium · toxicology

Introduction

Numerous tellurium-based drugs have been developed. For example, trichloro(dioxoethylene-O, O')-tellurate and 4,4'-di-hydroxydiphenyltelluride are used as enzyme inhibitors for cysteine proteases and as redox modulators for glutathione, respectively.[1] In addition, tellurite (TeO₃²⁻) ions possess antibiotic properties and have been used to inhibit the growth of many microorganisms, especially penicillin-insensitive bacteria.[2] However, the toxicity of TeO₃²⁻ ions released from Te-based drugs is a major safety concern.

Elemental Te is a p-type semiconductor with a band-gap energy of 0.35 eV, and thus Te nanomaterials (NMs), similar to TiO₂ and ZnO NMs, can be used as antibacterial agents. Although TiO₂ and ZnO NMs are effective, they are somewhat toxic to zebrafish,[3-5] Daphnia magna,[6-8] and mammalian cells.[9-14] Thus, preparation of safer NMs for use as antibacterial agents is still required. Based on the fact that Se nanoparticles (NPs) and selenite (SeO₃²⁻) ions have a similar bioavailability in rats, of which the former is much less acutely toxic in mice,[15] we expect that Te NMs may have similar antibiotic properties provided by TeO₃²⁻ ions, with a lower toxicity. Because of its unique helical-chain conformation, Te has a highly anisotropic growth tendency to form one-dimensional (1D) structures such as nanobelts,[16-19] nanowires,[20-22] and nanotubes[24-27] through van der Waals interactions in a hexagonal lattice. However, external energy is required for the preparation of these 1D Te structures.

In this study, we developed a facile approach for the preparation of Te NMs in an N₂H₄ solution (4m) containing 100 mM NaCl at 60°C (Figure 1d). We found that spherical Te nanoparticles (32 nm in diameter) with TeO₃²⁻ ions were formed in the E. coli cells. Compared to Ag nanoparticles that are commonly used as antibacterial reagents, Te NMs have higher antibacterial activity and lower toxicity. Thus, Te NMs hold great practical potential as a new and efficient antibacterial agent.

Results and Discussion

Synthesis of Te NMs

The TEM images depicted in Figure 1 indicate that various shapes and sizes of Te NM were synthesized from TeO₂ using N₂H₄ as a reducing agent under different reaction conditions. At 25°C, Te nanowires 20 (±3) nm in width and 880 (±170) nm in length were synthesized in the presence of 16m N₂H₄ (Figure 1a).[20] Te nanopencils with a width of 70 (±10) nm and a length of 440 (±70) nm were prepared in the presence of 8m N₂H₄ at 60°C (Figure 1b). High temperatures (higher collision rate) were unfavorable for the growth of long Te NMs.[26,27] Varying the concentration of N₂H₄ controlled the width and length of the Te nanopencils by changing their seeding and growth rates.[30-33] For example, at 4m N₂H₄, Te nanorices were obtained with a width and length of 50 (±8) nm and 130 (±20) nm, respectively (Figure 1c). The morphology of Te NMs was further varied by adding NaCl to the mixture. Te nanocubes with edge lengths of 80 (±20) nm were synthesized in an N₂H₄ solution (4m) containing 100 mM NaCl at 60°C (Figure 1d). In-
teractions of Cl⁻ ions with the specific facets of the nano-
crystal varied the order of free energies of different facets,
which significantly affected the relative growth rates of dif-
f erent facets and led to the formation of differently struc-
tured nanocrystals. Figure 2 shows the X-ray diffraction
(XRD) patterns of as-prepared Te NMs. The XRD patterns
reveal a high purity of as-prepared Te NMs, as assessed by
using bulk Te crystals with a trigonal structure as an index.
The intense diffraction peaks imply good crystallization of
the samples. Interestingly, the (101) diffraction peaks of the
Te nanorices and nanocubes (Figure 2c,d) are much stronger
than those of the Te nanowires and nanopencils. In addition,
the UV/Vis absorption spectra (Figure 3) vary among Te
NMs in aqueous solutions. A distinct absorption band of the
Te nanowires occurs at around 680 nm (curve a), which is
due to the transition from the valence band (p lone-pair
VB3) to the conduction band (p-antibonding CB1). Compared
to Te nanowires, the absorption bands of Te nanorices
and nanocubes (curves c and d) occur at smaller wave-
lengths mainly because their lengths are smaller. The spectra
reveal that the electronic transitions in Te NMs are sensitive
to their morphologies. The colors of the solutions containing
Te nanowires, nanopencils, nanorices, and nanocubes are
blue, green, brown, and orange, respectively. The Te NMs
shown were well dispersed in water (see Figure 3) and were
stable at 4°C for more than 8 months. We conducted induc-

tively coupled plasma–mass spectrometry (ICP-MS) and
energy-dispersive X-ray spectroscopy (EDX) measurements
to further confirm the purity of the as-synthesized Te NMs.
The ICP-MS data revealed an almost complete reduction of
TeO₂ to elemental Te to form these four Te NMs. The EDX
data revealed the absence of sulfur (from sodium dodecyl
sulfate used to terminate the synthesis of NMs and to stabi-
lize the as-prepared NMs) and nitrogen (from N₂H₄ or side
products) in the Te NMs. A very weak oxygen signal
(oxygen/tellurium ratio of 0.02) was detected, which likely
originated from copper oxidation and/or the formvar/carbon
film. A representative EDX spectrum of Te nanowires is
shown in Figure S6 in the Supporting Information.

**Antibacterial Activities of the Te NMs**

The antibacterial activities of the as-prepared Te NMs
against *E. coli* were examined using SYTO9 and propidium
iodide (PI), two dyes that both stain nucleic acids. While the
green-fluorescing dye SYTO9 is able to enter all cells, the
red-fluorescing PI is excluded from cells with structurally
intact cytoplasmic membranes. The optical microscopic
images (Figure 4a–d) show red stains in the *E. coli* cells
treated with the four differently shaped Te NMs and green
stains (Figure 4e) in the untreated *E. coli* cells, revealing
that the four Te NMs possessed strong antibacterial activity.
We note that these four Te NMs were stable for at least one
week in LB medium at 37°C (Figure S5 in the Supporting
Information), i.e., under the conditions used for antibacteri-
al tests. The percent viability values of the *E. coli* cells with/
without treatment with Te NMs were obtained from fluores-
cence measurements (see the Experimental Section) and are
plotted in Figure 4f. The live/dead ratio of cells in bacterial
populations was used as a measure of antibacterial activity
because the antibacterial tests were conducted in LB
medium, in which the bacteria are growing. Thus, the total
number of bacteria did not remain constant. The fluores-
cence approach allowed counting a large number of bacteri-
al cells at the same time, which provided more reliable data
than those obtained by counting the bacteria under the mi-
croscope. The microscopic images, however, do display the anti-
bacterial activity of Te NMs more directly. Treatment with
the four Te NMs (20 μg/mL) resulted in an E. coli live/dead ratio of less than 0.1. To distinguish the antibacterial activity of the four Te NMs, we reduced their concentrations to 10 μg/mL. Only about 30% of E. coli cells remained alive after treatment with Te nanowires and nanopencils, whereas only 15% were alive after treatment with the Te nanorices and nanocubes. As a control, the antibacterial activity of Ag NPs (10 nm in diameter; Figure S1 in the Supporting Information) was examined. After treatment with Ag NPs (10 μg/mL), 30% of E. coli cells remained alive; thus, these Ag NPs showed an antibacterial activity similar to those of the Te nanowires and nanopencils (10 μg/mL).

As a control, we tested the activity of Na₂TeO₃ that has found use as an antibacterial agent (Figure 4f). As N₂H₄, NaCl, and SDS may be adsorbed on the surface of Te NMs, we tested their effect on the growth of E. coli. It was confirmed that they exhibited negligible antibacterial activity at concentrations of 1 μM.

We then used LLC-PK1 cells (a renal cell line that can take up metal ions) to test the toxicity of Te NMs, sodium tellurite, and Ag NPs (Figure S2 in the Supporting Information). The results revealed that the toxicity of Te NMs was about 3–4 times lower than that of sodium tellurite and Ag NPs. Therefore, Te NMs may be safer than Ag NPs. The lower toxicity of the four Te NMs is owing to their higher stability in the cells and media. We suspected that the antibacterial activity of the four Te NMs stems from their oxidation in the media, which results in the formation of TeO₃²⁻ ions. These ions are toxic to most microorganisms, particularly gram-negative bacteria.

It has been shown that different Te colloids are formed through reduction of TeO₃²⁻ ions in LB and in M9 culture media, resulting in changes in the absorption spectra. The elongated Te colloids formed in the LB medium absorption at a larger wavelength (500 nm) than the spherical Te colloids formed in the M9 medium (320 nm). Different absorbance values at 500 nm reflect different amounts of TeO₃²⁻ ions produced in the media. The observed decrease in the absorbance value of nanocubes > nanorices > nanopencils > nanowires is consistent with the decreasing order of their antibacterial activity. In addition, we note that morphology-dependent antibacterial effects were observed using TiO₂, Ag₂O, and Ag NMs.

A representative TEM image (Figure 5) shows that Te NPs (32 nm in diameter) were formed inside the E. coli cells after incubation with Te nanowires. We point out that the formation of elemental Te nanoparticles was due to the reduction of released TeO₃²⁻ ions by thiol-containing molecules such as glutathione and cysteine, which are present inside the bacteria. We note that similar Te NPs were formed in the E. coli cells after treatment with the other three Te NMs prepared herein.

**Conclusions**

Four differently shaped Te NMs were prepared and tested as efficient antibacterial agents against E. coli. The four high-quality Te NMs were easily prepared by controlling the...
concentrations of N\textsubscript{2}H\textsubscript{4} and NaCl as well as temperature. The antibacterial activities of the four Te NMs against \textit{E. coli} are high (live/dead ratio < 0.1). Compared to Te nanowires, nanopencils, and Ag NPs, Te nanorices and nanocubes with a (101) crystal plane as the basal plane exhibited stronger antibacterial activities. The most exciting result is that the toxicity of Te NMs toward mammalian cells (LLC-PK1) is lower than that of Ag NPs. With high antibacterial activity and low toxicity toward normal cells, we strongly believe that Te NMs hold great potential for use as efficient antibacterial agents.

**Experimental Section**

**Materials**
Sodium dodecyl sulfate (SDS; > 98.5\%), silver nitrate (> 99\%), and titanium dioxide (Aeroxide P25) were obtained from Sigma-Aldrich. Sodium hydroxide (98.5\%) and sodium chloride (≥ 99\%) were obtained from Acros. Tellurium dioxide (99.9\%) and hydrazine monohydrate (80\%) were purchased from Showa. The BacLight Bacterial Viability and Counting Kit, RPMI-1640 medium, trypan blue, and AlamarBlue were acquired from Invitrogen. Fetal bovine serum (FBS), antibiotic–antimycotic solution, l-glutamine, and nonessential amino acids were purchased from BioWest. Ultrapure water from a Milli-Q ultrapure system (18.2 MΩcm) was used throughout this study.

**Synthesis of Te NMs**

Four differently shaped Te NMs were prepared through the reduction of tellurium dioxide with hydrazine. Te nanowires were typically prepared by adding, under constant magnetic stirring, hydrazine (16 m\textsubscript{L}) to a beaker containing tellurium dioxide powder (16 mg) at ambient temperature (25°C) over the course of 1 min. The mixture was reacted for 2 h. For the preparation Te NMs with other shapes, tellurium dioxide powder (16 mg) was dissolved in a sodium hydroxide solution (20 m\textsubscript{L}, 1 m\textsubscript{L}) to form soluble tellurite ions. Aliquots (5 and 2.5 m\textsubscript{L}) of hydrazine (16 m\textsubscript{L}) were dispensed in a 96-well plate and then 100 m\textsubscript{L} of the dye mixture was added to each well. The mixtures were incubated for 15 min at room temperature. Fluorescence intensities of SYTO 9 (excitation: 475 nm, emission: 530 nm) and PI (excitation: 475 nm, emission: 640 nm) were recorded separately. The green/red fluorescence ratio was used to calculate the percentage of live/dead cells.

**Cell Viability Assay**
Renal proximal tubular LLC-PK1 cells (American Type Culture Collection) were maintained in RPMI-1640 medium supplemented with 10% FBS, an antibiotic–antimycotic solution (1%), l-glutamine, and nonessential amino acids (1%). The cells were seeded in 96-well plates at an initial cell density of 1 × 10\textsuperscript{4} cells/mL\textsuperscript{−1}. The cell number was determined by the trypan blue exclusion method. After incubation with Te NMs and Ag NPs (24 h), cell viability was assayed using AlamarBlue.

**Acknowledgements**

This study was supported by the National Science Council of Taiwan under the contract NSC 98-2113M-002-011-MY3.

