JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

Characterization, Antimicrobial Activity, and Mechanism of a High-Performance (−)-Epigallocatechin-3-gallate (EGCG)−Cu^{II}/Polyvinyl Alcohol (PVA) Nanofibrous Membrane

Li-ming Sun,[†] Chen-lu Zhang,[‡] and Ping Li*[,†,§]

[†]School of Life Sciences and Technology, Tongji University, Shanghai 200092, People's Republic of China

[‡]College of Life Sciences, Nanjing Agricultural University, Nanjing 210095, People's Republic of China

[§]Shanghai Key Laboratory of Molecular Catalysis and Innovative Materials, Department of Chemistry, Fudan University, Shanghai 200433, People's Republic of China

ABSTRACT: (−)-Epigallocatechin-3-gallate (EGCG) exhibited strong antimicrobial activity. However, the easy oxidation of EGCG has limited its application. To increase the antimicrobial activity and stability of EGCG, the EGCG−Cu^{II} complex was formed by chelating copper ions and then electronspun into polyvinyl alcohol (PVA) nanofibers. Electronspun nanofibrous membranes were investigated by Fourier transform infrared (FTIR) spectroscopy and scanning electron microscopy (SEM), which showed that the average fiber diameter was 210 nm. Minimal inhibitory concentrations (MICs) of EGCG−Cu^{II}/PVA membranes were tested against the tested strains. The bactericidal activity of EGCG−Cu^{II} was suppressed by ethylenediaminetetraacetic acid (EDTA). Cell killing was accompanied by the leakage of intracellular proteins, indicating that the cytoplasmic membrane was badly damaged after exposure to the EGCG−Cu^{II}/PVA membrane. We observed the process of cell damage by SEM. On the basis of experimental evidence and theoretical analyses, the mechanism proposed that copper ions played a cooperative role in the bactericidal process of EGCG. To evaluate the antimicrobial activity of the EGCG−Cu^{II}/PVA membrane, we developed a rapid detection method by labeling cells with water-soluble CdTe quantum dots.

KEYWORDS: Antimicrobial activity, electrospinning, EGCG, Cu^{II}, CdTe QDs, PVA

INTRODUCTION

The tea catechins have been shown to possess antibacterial or bactericidal activities.[1−5] (−)-Epigallocatechin-3-gallate (EGCG), one of the major components of catechins, is a naturally broad-spectrum antimicrobial agent. Bacterial species has been described included *Escherichia coli*, *Stenotrophomonas maltophilia*, *Bacillus* bacteria, *Listeria monocytogenes*, and *Staphylococcus aureus*.[6−10] Ikigai et al. reported that the minimum inhibitory concentration (MIC) of EGCG against *E. coli* K-12 was 1.25 mmol L^{-1}, while the bactericidal activity against Gram-negative bacteria is much lower than that against Gram-positive bacteria.[11] There are only a few reports on the process and mechanism of cell damage after exposure to EGCG.

Because EGCG is unstably prone to the oxidation−reduction reaction, the application of EGCG is limited. One solution is to immobilize it into an appropriate carrier, which would be very important for its activity and stability. The use of electrospun polyvinyl alcohol (PVA) nanofibers as a carrier has been popular because of its unique nanoeffects and biocompatibility, which have been especially used in different fields, such as biomedicine, biocatalyst, tissue engineering, and other industrial fields.[12−14] However, thus far, there are no reports of electrospinning EGCG or its modification for nanomaterial preparation and function.

The rapid evaluation plays an important role in the antimicrobial reagent applications. Standard plate count (SPC), a traditional evaluation method, requires time-consuming preparation, usually taking 24−48 h. Fluorescence analysis is a rapid, technically simple, and efficient method for directly detecting the target microorganism from samples. Quantum dots (QDs), as novel fluorescent markers, have long-term photostability, high quantum yield, broad absorption spectra, and narrow, symmetric emission.[15,16] Several studies indicated that QDs could link with biorecognition molecules, such as proteins and peptides, on the cell surface, which was then applied to biological analysis and imaging.[17,18]

In this paper, aqueous solutions of PVA containing the EGCG−Cu^{II} complex were fabricated into a nanofibrous membrane by electrospinning and the membrane was characterized using Fourier transform infrared (FTIR) spectroscopy and scanning electron microscopy (SEM). The EGCG−Cu^{II}/PVA fibrous membrane was proven to have good antimicrobial activity. Meanwhile, we developed a rapid, simple, and highly sensitive detection method to evaluate the antimicrobial activity of the resulting nanofibrous membrane.

MATERIALS AND METHODS

Materials. EGCG was purchased from Sigma (Shanghai, China). PVA powder with a polymerization degree of 1750 ± 50 was obtained from Sinopharm Group Chemical Reagent Co., Ltd. (Shanghai, China). 1-Ethyl-3-(3-dimethyllaminopropyl)-carbodiimide (EDC) was obtained from Shanghai Mychems Company. The strains used in this study were from our laboratory. All other chemicals were made in China and of analytical grade or molecular biology grade.

Received: February 10, 2011
Accepted: March 21, 2011
Revised: March 19, 2011
Published: March 21, 2011

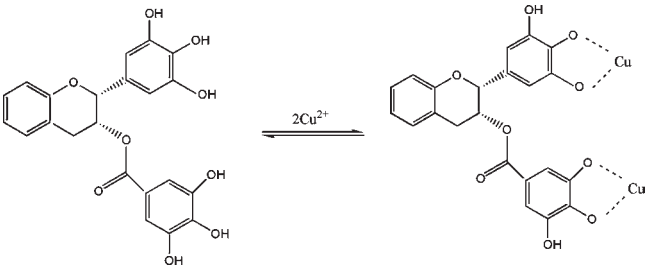

Figure 1. Complexation reaction between EGCG and Cu^{II}.

Preparation of the EGCG−Cu^{II} Complex. EGCG aqueous solution (0.5 mmol L^{-1}) was incubated with $CuSO_4$ (1 μmol L^{-1} dissolved in dH_2O) for 10 min, forming a complex with Cu^{II} at room temperature (Figure 1). The modifications of the absorption spectra of EGCG solutions when combined with Cu^{II}, at different pH values, were analyzed with a Varian Cary 50 Probe ultraviolet−visible (UV−vis) spectrophotometer (Varian, Palo Alto, CA).

Preparation of the EGCG−Cu^{II}/PVA Nanofibrous Membrane. Stock solution of EGCG (5×10^{-3} mol L^{-1}) was prepared and mixed with 8% (w/v) PVA. The mixture was fabricated into nanofibrous membranes, with the final concentration of Cu^{II} constant at 1 μmol L^{-1}, which is lower than the toxic concentration of 1 mg L^{-1}. A syringe pump (WZ-50C2, Zhejiang University Medical Instrument Co., Ltd., China) was used to feed the mixture solution through a 20 mL plastic syringe fitted with a needle of tip diameter of 1.2 mm, which was connected with a high-voltage power supply (GDW-A, Beijing Institute of High Voltage Electrical and Mechanical Technology, Inc., China) for electrospining. The flow rate was 1 mL h^{-1}. The strength of the electrostatic field was 16 kV. The mixture solution formed from the Taylor cone and sprayed to a grounded collector, approximately 6 cm from the needle tip. All of the experiments were performed at room temperature (~25 °C), with a humidity of about 50%.

The morphology of EGCG−Cu^{II}/PVA fibrous membranes was characterized by Nicolet 6700 Fourier transform infrared spectroscopy (FTIR, Thermo Nicolet Corporation, Madison, WI). The membrane was observed with a XL30 scanning electron microscope (Philips, Holland) after gold sputter-coating.

MIC Estimation. All strains were cultured routinely, harvested by centrifugation (8000*g* at 4 °C for 10 min), washed twice in phosphate-buffered saline (PBS, 0.2 mol L^{-1} at pH 7.4), and resuspended at a final concentration of approximately 10^7 cells/mL of PBS. To determine the MIC,[19,20] the resuspended cells were inoculated into fresh medium supplemented with various concentrations of the membrane and grown overnight at 37 °C (or 30 °C) and then the minimum concentration of the membrane giving cultures that did not become turbid was taken to be the MIC. The determination of the MIC for each isolate was carried out in triplicate, and the results were taken when there was agreement in at least two of the three MIC results. Control experiments were performed with sterile PVA fibrous membrane only.

Experimental Determination of the Cooperative Role of Copper Ions. After incubation of *E. coli* with (or without) EGCG in the presence of Cu^{II} at 37 °C, the supernatant fluid was separated by centrifugation (8000*g* at 4 °C for 10 min). The copper concentration in the supernatant fluid was determined by an Agilent 3510 atomic absorption spectrometer (Agilent Technologies, Shanghai, China). As for inhibition experiments, ethylenediaminetetraacetic acid (EDTA) was added to the cell suspensions containing EGCG−Cu^{II} and the samples of the solution were withdrawn at various time intervals. After additional serial dilution in PBS, the viable cell number was determined by a standard plate assay on Luria−Bertani (LB) agar.

Analysis of the Protein Content in the Supernatant of the Cell Culture. To examine the protein level changes in the supernatant after exposure to the membrane containing the EGCG−Cu^{II} complex, a Libra S22 spectrometer (Biochrom, Ltd., Cambridge, U.K.) was used.

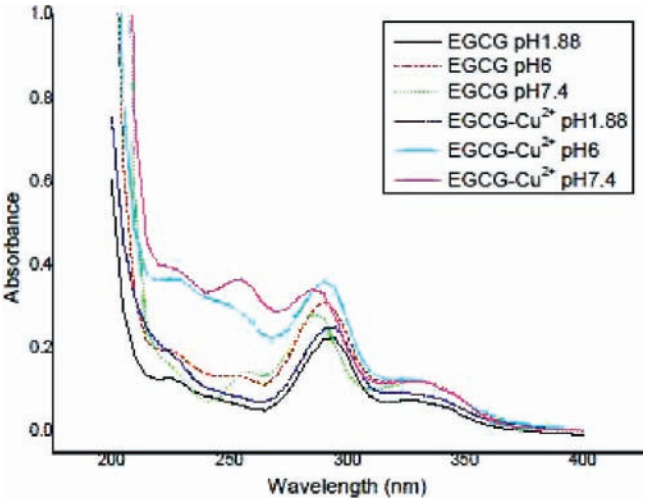

Figure 2. UV spectra for EGCG (0.5 mmol L^{-1}) with or without $CuSO_4$ (1 μmol L^{-1}).

SEM Imaging of Cell Morphologies at Different Times. Cells were treated with the EGCG−Cu^{II}/PVA nanofibrous membrane, then collected by centrifugation, washed twice, and resuspended in PBS. The cells were fixed for 2 h in 2.5% glutaraldehyde at room temperature (~25 °C), dehydrated, and then coated with gold. The interactions of the nanofibrous membranes with bacterial cells at different times were examined using SEM.

Synthesis and Fluorescence Spectra of CdTe QDs. The preparation of water-soluble CdTe QD solution was described in ref 21 using the reaction between Cd^{2+} and NaHTe solution, with thioglycolic acid (TGA) as the stabilizer. Absorption spectra were obtained using a F-2500 fluorescence spectrophotometer (Hitachi High-Technologies Corporation, Tokyo, Japan). Fluorescence intensity and emission spectra of CdTe QDs coupled with microorganism cells were recorded with the excitation wavelength fixed at 400 nm.

CdTe QDs Coupled with the Tested Strain. Water-soluble CdTe QDs were conjugated to the tested cells using EDC (0.1 mmol L^{-1}) as a coupling reagent for 1 h. Then, cells were purified using the ultrafiltration membrane (0.22 μm) to wash away broken cell debris and the excess CdTe QDs.

Fluorescence Microscopy Analysis. The effects of the EGCG−Cu^{II}/PVA membrane on the tested strain were monitored using Olympus BX51TRF microscopy (Olympus Corporation, Tokyo, Japan). Drops (5 μL) of microbial cultures were placed on microscope slides and observed under the microscope with an Olympus DP72 camera (Olympus Corporation, Tokyo, Japan).

RESULTS AND DISCUSSION

Effect of pH on the Spectrum of EGCG−Cu^{II}. Miller and Weber reported that flavonoids containing a 3,4-dihydroxy structure and trihydroxy gallate structure could chelate copper.[22,23] In their studies, EGCG formed a stable complex with Cu^{II} by a gallate moiety. The structure acted as a bidentate chelating agent, where two EGCG molecules can coordinate with Cu^{II} in one plane.[24] As shown in Figure 2, the spectra of EGCG solution (pH 7.4) changed significantly in the presence of copper ions, which demonstrated that EGCG formed a complex with Cu^{II} in PBS. These complexes would dissociate under acidic conditions (pH 1.88), releasing free EGCG. The results of these spectroscopic

experiments supported the observation that EGCG could chelate copper ions.

Characterization of the EGCG−Cu^{II}/PVA Nanofibrous Membrane. Electrospinning has proven to be a powerful means of fabricating polymer nanofibers, but its applicability to obtain a uniform, smooth fibrous structure is not straightforward. Among various parameters of the electrospinning process, the concentration or viscosity of spinning solution, solution delivery rate, applied voltage, and needle tip−receiver distance were the most effective variables for controlling fiber morphology and diameter. Results indicated that the solution concentration was the major factor controlling the morphology of the fibers in the electrospinning of PVA. Smooth and homogeneous fibers without beads were produced when the PVA concentration reached 8%.[25] Figure 3 showed that the morphologies of PVA fibrous membranes only changed slightly, and smooth fibers could be obtained when the EGCG−Cu^{II} concentration was below 1 mmol L^{-1}. The results also indicated that the average diameters of the fibers were basically unchanged with an increasing concentration of EGCG−Cu^{II}. The average diameter of the fibers was 210 nm.

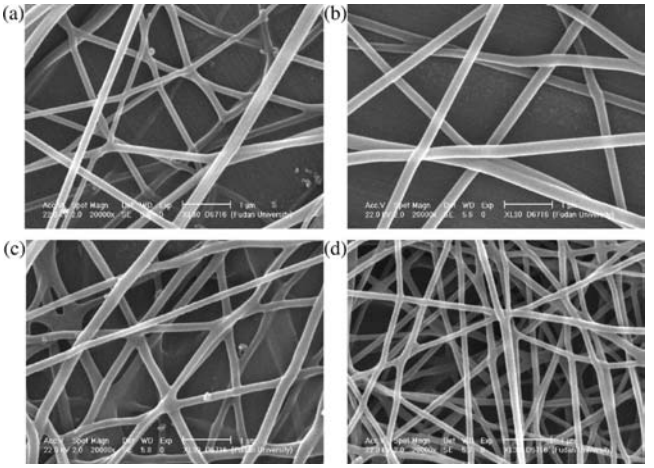

Figure 3. SEM images of EGCG−Cu^{II}/PVA fibrous membranes. PVA fibrous membranes were electrospun from 8% (w/v) solution at various concentrations of EGCG−Cu^{II}: (a) 0.025 mmol L^{-1}, (b) 0.05 mmol L^{-1}, (c) 0.5 mmol L^{-1}, and (d) 1 mmol L^{-1}.

In Figure 4, the peak at 2360 cm^{-1} was assigned to the stretching vibration of the cumulated double bond. In addition, multi-peaks at 1600, 1580, and 1500 cm^{-1} were also found in the spectrum, associated with the stretching vibration of heteroaromatics. These characteristic peaks showed that EGCG−Cu^{II} dispersed uniformly in the PVA fibrous membrane.

MICs of EGCG−Cu^{II}/PVA Membranes. In previous experiments, the EGCG−Cu^{II}/PVA nanofibrous membrane showed better efficacy to the tested strains with a longer duration effect than the EGCG−Cu^{II} complex only. The antimicrobial activities of the EGCG−Cu^{II}/PVA membrane against the tested strains were investigated, with the MICs presented in Table 1. The

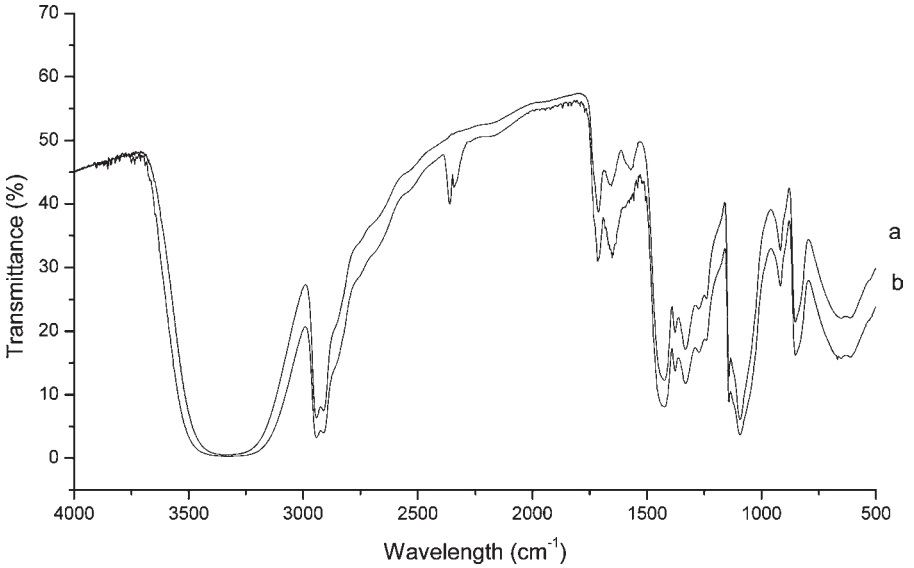

Figure 4. IR spectra of PVA (curve a) and the EGCG−Cu^{II}/PVA fibrous membrane (curve b).

Table 1. Comparison of the Mean MIC among Microbial Groups

	mean MIC ± standard deviation (μmol L^{-1})		
test organism	EGCG−Cu^{II}/PVA membrane	EGCG−Cu^{II} complex	temperature (°C)
Bacillus cereuse	8 ± 3	10 ± 5	30
Bacillus subtilis	28 ± 9	25 ± 11	30
Staphylococcus aureus	200 ± 55	250 ± 89	37
Escherichia coli	45 ± 18	50 ± 33	37
Pseudomonas nitroreducens	20 ± 8	25 ± 7	30
Saccharomyces cerevisiae	500 ± 142	450 ± 157	30
Candida albicans	600 ± 217	500 ± 123	30

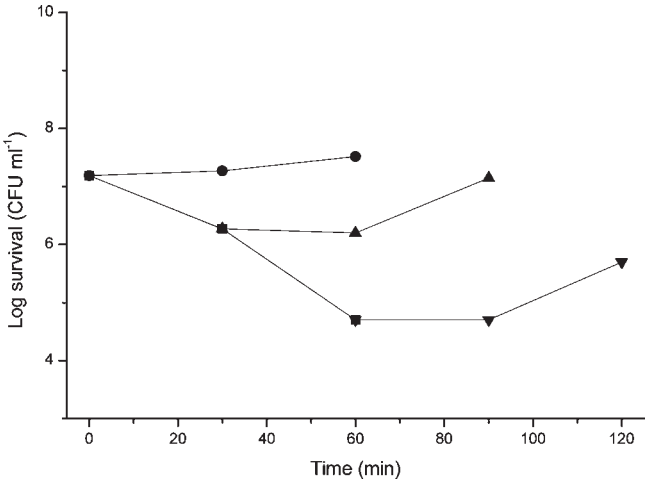

Figure 5. Inhibitory effect of EDTA on the bactericidal activity of EGCG−Cu^{II}. EGCG (0.5 mmol L^{-1}) and then Cu^{II} (1 μmol L^{-1}) were added to the washed *E. coli* cells. After incubation of cells in PBS (pH 7.4) at 37 °C, cells were withdrawn at various time internals and viable cell numbers were determined (■). EDTA (2 mmol L^{-1}) was added to the cell suspension at 2 min (●), 30 min (▲), and 60 min (▼).

bactericidal activity against Gram-negative bacteria was not significantly weaker than that against Gram-positive bacteria. The activity against *S. aureus* (Gram-positive bacteria) was lower than that against other bacteria (including Gram-negative bacteria) in Table 1. The fungicidal activity was also examined.

Determination of the Cooperative Effect between EGCG and Copper Ions. The percentages of copper ions bound to the cells increased in the presence of EGCG (data not shown). Meanwhile, EGCG caused exponential killing of *E. coli* cells in the presence of Cu^{II}. However, this bactericidal activity of EGCG−Cu^{II} was inhibited by EDTA (a strong chelating agent), because the addition of EDTA to the cell suspensions after 2, 30, and 60 min of incubation completely inhibited additional killing (Figure 5). It showed that the copper ions played a cooperative role in the antimicrobial process of EGCG against *E. coli*.

Evaluation of Protein Leakage. We used spectrometry to detect the change of the protein content in the supernatant of the cell culture after exposure to the EGCG−Cu^{II}/PVA membrane. Standard curves, which had been prepared with PBS containing Coomassie Brilliant Blue G250 and bovine serum albumin (BSA), were used for calibration (see Figure 6a). With increasing EGCG−Cu^{II} content in the fibrous membrane, the protein level in the supernatant of the cell culture was found to increase (see Figure 6b). When the concentration of the EGCG−Cu^{II} complex in the fibrous membrane was 0.5 mmol L^{-1}, the protein level in the supernatant of the cell culture was 57.5 μg mL^{-1}. Thus, cells were more disrupted with an increasing EGCG−Cu^{II} content, causing the leakage of intracellular materials.

Morphologies of Damage Cells Treated by the EGCG−Cu^{II}/PVA Membrane. Several studies showed that high concentrations of EGCG were toxic to cells. Ikigai et al. reported that tea polyphenol (TPP) damaged bacterial membranes and the bactericidal effect of EGCG was attributed to membrane perturbation.[11] To assess this, we observed the morphological changes of microbial cells exposed to the EGCG−Cu^{II}/PVA membrane. The morphological changes of cells can be seen clearly from the SEM images shown in Figures 7 and 8, where normal *E. coli* cells grown in LB medium in the presence of the sterile PVA fibrous membrane only

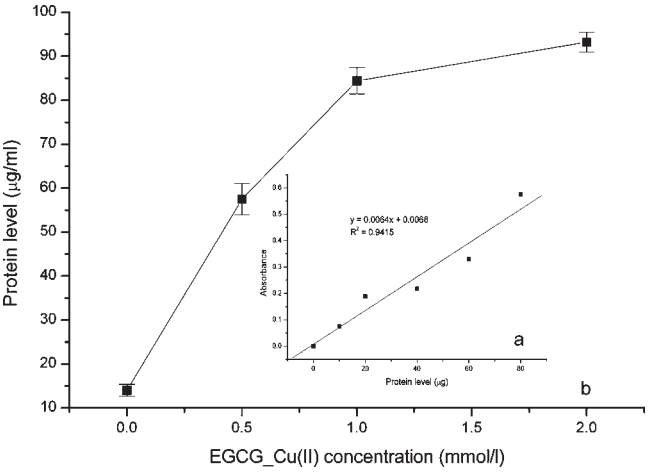

Figure 6. Standard curve and effect of different concentrations of EGCG−Cu^{II} on the protein level in the supernatant.

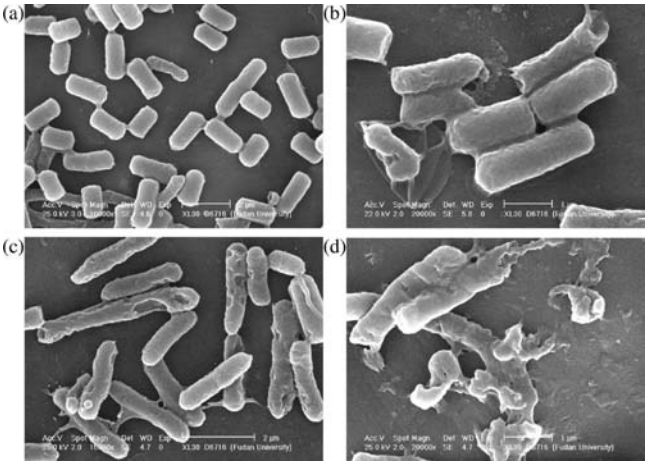

Figure 7. Scanning electron micrographs of *E. coli* either untreated (a) or treated with the fibrous membrane containing 0.5 mmol L^{-1} EGCG−Cu^{II} for 2, 4, and 6 h (b−d).

exhibited a typical rod shape with a smooth surface (Figure 7a). However, cells treated with the membrane containing 0.5 mmol L^{-1} EGCG−Cu^{II} showed several destructive openings on the cell envelope as well as a preponderance of irregular rod forms with wrinkled surfaces (panels b−d of Figure 7). The morphological changes of *S. cerevisiae* exposed to the EGCG−Cu^{II}/PVA membrane were displayed in Figure 8.

Spectrum of Water-Soluble CdTe QDs. Figure 9 showed the absorption spectrum curve a and emission spectra curve b of typical CdTe QDs used in *S. cerevisiae* cells labeled in this study. The absorption spectrum indicated that CdTe QDs had a wider range of absorption, with peaks at 400 and 460 nm. The emission spectrum was characterized by good symmetry and relatively narrow spectral width, with the peak at 548 nm. The results indicated that CdTe QDs displayed high quantum yield and good water solubility and photostability, which was similar to the reports in ref 16.

Fluorescence Detection of the Total Count of *S. cerevisiae*. QDs, as a novel fluorescent marker, have been applied to cell imaging and analysis. Hirschey et al. imaged *E. coli* with water-soluble CdTe QDs.[18] In the previous experiments, we successfully labled the tested strains (in Table 1) with water-soluble

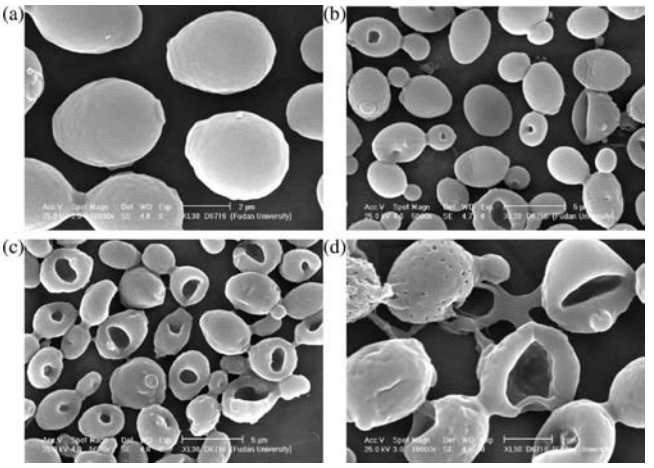

Figure 8. Scanning electron micrographs of *S. cerevisiae* either untreated (a) or treated with the fibrous membrane containing 0.5 mmol L^{-1} EGCG−Cu^{II} for 2, 4, and 6 h (b−d).

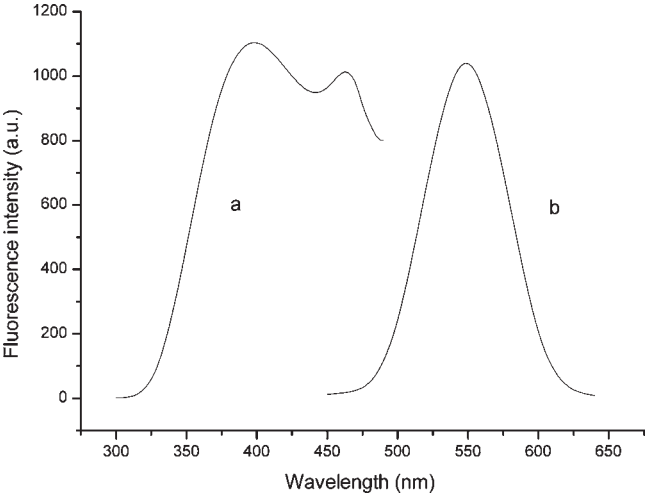

Figure 9. Absorption spectra (curve a) and fluorescence emission spectra (curve b) of CdTe QDs.

CdTe QDs. Thus far, relatively little was known whether QDs could be feasible in detecting fungal cell counts. However, here, we found that the fluorescence intensity rose with an increasing cell count in the range of 10^3−10^7 colony forming units (CFU)/mL of *S. cerevisiae* cells (Figure 10). The relationship between fluorescence signal intensity and log total cell count followed the equation $y = 51.286x + 51.886$ ($R^2 = 0.9888$) over the range of 10^3−10^7 CFU/mL. The number of cells detected by fluorescence signal intensity was slightly higher than that by standard plate count (SPC) in this study. The method of QD labeling was less time-consuming than that of SPC, usually taking 1−2 h. Figure 11 showed the fluorescence images of 10^3−10^7 CFU/mL of *S. cerevisiae* cells.

Antimicrobial Mechanism of the EGCG−Cu^{II}/PVA Membrane. In the previous experiments, we found that the percentages of survived *E. coli* cells incubated with the increase of EGCG decreased significantly in the presence of 1 μmol L^{-1} Cu^{II}. Meanwhile, 1 μmol L^{-1} Cu^{II} showed no effect on the viability of the cells incubated in PBS buffer at 37 °C for 60 min. The assessment for the antimicrobial activity of the nanofibrous membrane against the tested strains showed an enhanced activity

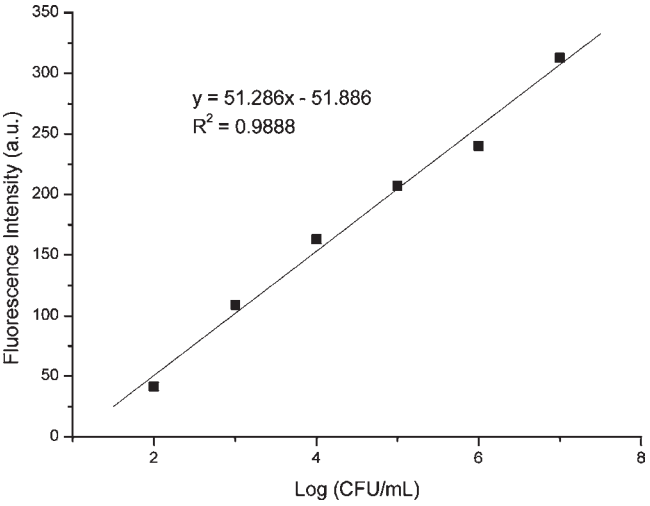

Figure 10. Effect of the count of *S. cerevisiae* on the fluorescence signal intensity.

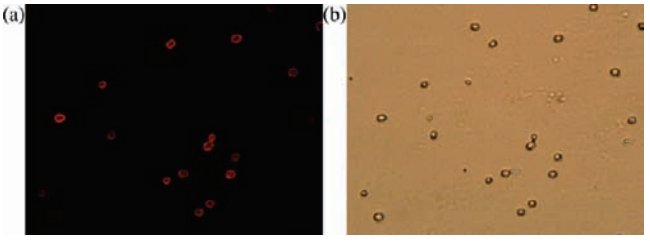

Figure 11. Fluorescence images of 10^4 CFU/mL of *S. cerevisiae* cells coupled with CdTe QDs (1000×).

with the increase of the EGCG−Cu^{II} content. The effective concentration of the EGCG−Cu^{II} complex in the membrane was higher than 0.05 mmol L^{-1}. The bactericidal activity of the EGCG−Cu^{II}/PVA membrane was inhibited by EDTA, a specific chelator of copper ion (Figure 5). This result indicated that the copper ions had a cooperative effect on the antimicrobial process of EGCG.

SEM images showed no significant change of the membrane morphology with the increase of the EGCG−Cu^{II} content. The interstitial spaces of the membranes were large enough for some microorganisms to penetrate to obtain full access to the EGCG−Cu^{II} complexes (Figure 3). Thus, the opportunity was greatly increased to contact the cells.

The antibacterial property of catechins has been explained by the suggestion that catechins exert an effect on the bacterial membrane resulting in the leakage of intracellular materials. Ikigai et al. reported that Gram-negative bacteria, such as *E. coli*, have negatively charged lipopolysaccharides on their cell surfaces.[11] At physiological pH, EGCG is uncharged but the resultant EGCG−Cu^{II} complex is considered to be positively charged. The negative charges on the cell surface of bacteria may attract the EGCG−Cu^{II} complex and result in an increase of the bactericidal activity of EGCG against bacteria. Atomic absorption proved that the copper ions were bound to the cells in the presence of EGCG. Additionally, it is known that Cu^{II} is complexed by EGCG and EGCG is apt to be present on microbial membranes.[20] We suggested that the oxidation−reduction reaction between Cu^{II} and Cu^{I} on the cell surface must be involved in the killing of cells.

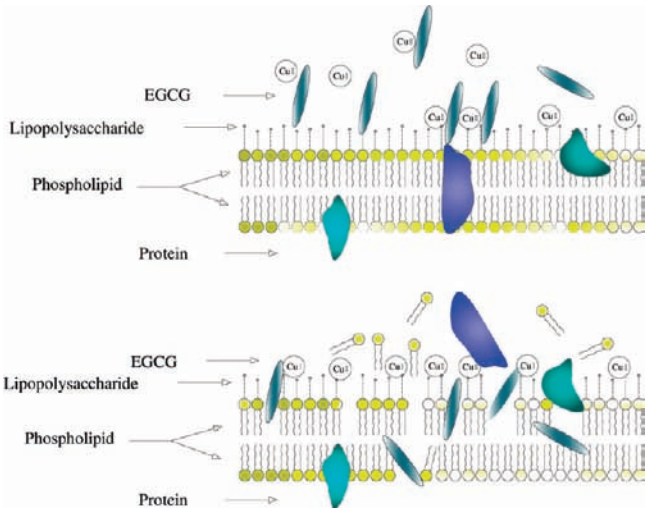

Figure 12. Schematic illustration of the cooperative effect of copper ions on the antimicrobial process of EGCG against *E. coli*.

Therefore, a mechanism was proposed that the reoxidation of Cu^{II} to Cu^{I} involving EGCG on the cell surface was responsible for the bactericidal activity of the nanofibrous membrane (Figure 12).

AUTHOR INFORMATION

Corresponding Author

*Address: School of Life Sciences and Technology, Tongji University, No. 1239 Siping Road, Shanghai 200092, China. Telephone: 86-21-65981051. Fax: 86-21-65988653. E-mail: liping01@tongji.edu.cn.

Funding Sources

This work was supported by the National Natural Science Foundation of China (20976138), the Natural Science Foundation of Shanghai (09ZR1434500), the Ministry of Agriculture of China (2009ZX08009-37B), and the Shanghai Key Laboratory of Molecular Catalysis and Innovative Materials (2010MCIMKF03).

REFERENCES

(1) Chacko, S. M.; Thambi, P. T.; Kuttan, R.; Nishigaki, I. Beneficial effects of green tea: A literature review. *Chin. Med.* **2010**, *5*, 13.

(2) Friedman, M. Overview of antibacterial, antitoxin, antiviral, and antifungal activities of tea flavonoids and teas. *Mol. Nutr. Food Res.* **2007**, *51*, 116–134.

(3) Hamilton-Miller, J. M. T. Antimicrobial properties of tea (*Camellia sinensis* L.). *Antimicrob. Agents Chemother.* **1995**, *39*, 2375–2377.

(4) Taylor, P. W.; Hamilton-Miller, J. M. T.; Stapleton, P. D. Antimicrobial properties of green tea catechins. *Food. Sci. Technol. Bull.* **2005**, *2*, 71–81.

(5) Toda, M.; Okubo, S.; Hiyoshi, R.; Shimamura, T. The bactericidal activity of tea and coffee. *Lett. Appl. Microbiol.* **1989**, *8*, 123–125.

(6) Navarro-Martinez, M. D.; Navarro-Peran, E.; Cabezas-Herrera, J.; Ruiz-Gomez, J.; Garcia-Canovas, F.; Rodriguez-Lopez, J. N. Antifolate activity of epigallocatechin gallate against *Stenotrophomonas maltophilia*. *Antimicrob. Agents Chemother.* **2005**, *49*, 2914–2920.

(7) Sakanaka, S.; Juneja, L. R.; Taniguchi, M. Antimicrobial effects of green tea polyphenols on thermophilic spore-forming bacteria. *J. Biosci. Bioeng.* **2000**, *90*, 81–85.

(8) Kohda, C.; Yanagawa, Y.; Shimamura, T. Epigallocatechin gallate inhibits intracellular survival of *Listeria monocytogenes* in macrophages. *Biochem. Biophys. Res. Commun.* **2008**, *365*, 310–315.

(9) Stapleton, P. D.; Shah, S.; Anderson, J. C.; Hara, Y.; Hamilton-Miller, J. M. T.; Taylor, P. W. Modulation of β-lactam resistance in *Staphylococcus aureus* by catechins and gallates. *Int. J. Antimicrob. Agents* **2004**, *23*, 462–467.

(10) Anderson, J. C.; Headley, C.; Stapleton, P. D.; Taylor, P. W. Synthesis and antibacterial activity of hydrolytically stable (−)-epicatechin gallate analogues for the modulation of β-lactam resistance in *Staphylococcus aureus*. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2633–2635.

(11) Ikigai, H.; Nakae, T.; Hara, Y.; Shimamura, T. Bactericidal catechins damage the lipid bilayer. *Biochim. Biophys. Acta, Biomembr.* **1993**, *1147*, 132–136.

(12) Bandyopadhyay, A.; Sarkar, M. D.; Bhownick, A. K. Poly(vinyl alcohol)/silica hybrid nanocomposites by sol−gel technique: Synthesis and properties. *J. Mater. Sci.* **2005**, *40*, 5233–5241.

(13) Bryaskova, R.; Pencheva, D.; Kale, G. M.; Lad, U.; Kantardjiev, T. Synthesis, characterisation and antibacterial activity of PVA TEOS Ag−Np hybrid thin films. *J. Colloid Interface Sci.* **2010**, *349*, 77–85.

(14) Huang, X. J.; Yu, A. G.; Jiang, J.; Pan, C.; Qian, J. W.; Xu, Z. K. Surface modification of nanofibrous poly(acrylonitrile-*co*-acrylic acid) membrane with biomacromolecules for lipase immobilization. *J. Mol. Catal. B: Enzym.* **2009**, *57*, 250–256.

(15) Leptinhn, S.; Har, J. Y.; Chen, J. Z.; Ho, B.; Wohland, T.; Ding, J. L. Single molecule resolution of the antimicrobial action of quantum dot-labeled sushi peptide on live bacteria. *BMC Biol.* **2009**, *7*, 1–13.

(16) Liu, Y. F.; Yu, J. S. Selective synthesis of CdTe and high luminescence CdTe/CdS quantum dots: The effect of ligands. *J. Colloid Interface Sci.* **2009**, *333*, 690–698.

(17) Duong, H. D.; Rhee, J. I. Use of CdSe/ZnS core−shell quantum dots as energy transfer donors in sensing glucose. *Talanta* **2007**, *73*, 899–905.

(18) Hirschey, M. D.; Han, Y. J.; Stucky, G. D.; Butler, A. Imaging *Escherichia coli* using functionalized core/shell CdSe/CdS quantum dots. *J. Biol. Inorg. Chem.* **2006**, *11*, 663–669.

(19) Sanpui, P.; Murugadoss, A.; Prasad, P. V. D.; Ghosh, S. S.; Chattopadhyay, A. The antibacterial properties of a novel chitosan−Ag-nanoparticle composite. *Int. J. Food. Microbiol.* **2008**, *124*, 142–146.

(20) Wong, S. Y. Y.; Grant, I. R.; Friedman, M.; Elliott, C. T.; Situ, C. Antibacterial activities of naturally occurring compounds against *Mycobacterium avium* subsp. *paratuberculosis*. *Appl. Environ. Microb.* **2008**, *74*, 5986–5990.

(21) Weng, J. F.; Song, X. T.; Li, L.; Qian, H. F.; Chen, K. Y.; Xu, X. M.; Cao, C. X.; Ren, J. C. Highly luminescent CdTe quantum dots prepared in aqueous phase as an alternative fluorescent probe for cell imaging. *Talanta* **2006**, *70*, 397–402.

(22) Miller, N. J.; Castelluccio, C.; Tijburg, L.; Rice-Evan, C. The antioxidant properties of theaflavins and their gallate esters—Radical scavengers or metal chelators. *FEBS Lett.* **1996**, *392*, 40–44.

(23) Weber, G. HPLC with electrochemical detection of metal−flavonoid-complexes isolated from food. *Chromatographia* **1988**, *26*, 133–138.

(24) Yoshioka, H.; Senba, Y.; Saito, K.; Kimura, T.; Hayakawa, F. Spin-trapping study on the hydroxyl radical formed from a tea catechin−Cu(II) system. *Biosci., Biotechnol., Biochem.* **2001**, *65*, 1697–1706.

(25) Yao, C.; Li, X. S.; Neoh, K. G.; Shi, Z. L.; Kang, E. T. Surface modification and antibacterial activity of electrospun polyurethane fibrous membranes with quaternary ammonium moieties. *J. Membr. Sci.* **2008**, *320*, 259–267.