Chapter 15
Nanomaterial-Based Antibacterial Paper

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15.1 Synthesis and Characterization of Nanomaterial-Based Films

The nanomaterial-based film with antibacterial property can be fabricated by physical and chemical methods. Physical methods include spin-coating, dip-coating, vacuum filtration, electrospinning, and magnetron sputtering.

15.1.1 Spin-Coating

Spin-coating is applied to prepare uniform thin film by spreading the solution placed on the flat substrates at a constant rate. The thickness of the film is determined by centrifugal forces controlled by spin speed, solution viscosity, and spin time. TiO$_2$ film and silver nanoparticles (AgNPs)/TiO$_2$ composite film have been prepared by spin-coating technology.

TiO$_2$ nanoparticles have been synthesized using the sol–gel method [2]. In a typical experiment, TiO$_2$ sol was prepared from the hydrolysis of Ti(OC$_4$H$_9$)$_4$: 0.01 mol of Ti(OC$_4$H$_9$)$_4$ stirred in ice incubation was mixed with 0.15 mol of ethanol. The ethanol/H$_2$O/acetylacetone solution (0.1 mol ethanol, 0.02 mol deionized water, 0.01 mol acetylacetone) was added to the Ti(OC$_4$H$_9$)$_4$/ethanol solution under stirring in ice incubation, then the mixture was stirred for 2 h. The TiO$_2$ sol was matured for about 48 h before coating. TiO$_2$ thin films were coated on titanium plate by the sol–gel spin-coating method with a rotating speed of 2,000 rpm. The resulting films were subjected to heat treatment at 200°C for...
By repeating this process, TiO$_2$ thin films with different thicknesses were obtained. Finally, the films were annealed at 500°C for 3 h in air for crystallization. The crystal structure and thickness of TiO$_2$ film were characterized by scanning electron microscopy (SEM) (Fig. 15.1) [3].

Zhang’s [4] and Yang’s [5] groups synthesized AgNPs/TiO$_2$ composite film according to the sol–gel spin-coating method. The SEM images and XRD suggested that the crystallinity and growth of AgNPs were improved by increasing the annealing temperature. Furthermore, the morphology of the AgNPs/TiO$_2$ composite film could be controlled by simply tuning the molar ratio of the silver nitrate, implying the morphology of composite film became rougher and rougher with the increase in the concentration of silver nitrate, while the diameter of AgNPs decreased. When the molar ratio of Ti$^{4+}$ to Ag$^+$ reached 5:1, the composite films were mesoporous. However, the AgNPs attached to the surface of TiO$_2$ nanoparticles by forming Ag-O-Ti bonds, rather than entering the lattice of the TiO$_2$ anatase phase [6].

15.1.2 Dip-Coating

The dip-coating method is a simple way to deposit thin film on the substrate. During the process, completely automated by computerized control system, the substrate is slowly dipped into and withdrawn from a tank containing the sol, with a uniform velocity, in order to obtain a uniform film. The film thickness is sensitive to flow conditions in the liquid bath and gas overhead, and is determined by the competition among viscous force, surface tension force and gravity. The faster the substrate was withdrawn, the thicker the film deposited. Many silver nanoparticles (AgNPs)-based films have been fabricated on glass and silica substrates with this procedure [7–10].

Zhang and co-workers [8] have reported a facile two-step method for the preparation of surface-silvered polymer films. The commercial polyimide (PI) film was functionalized by simply dipping the film into dopamine (DOPA) aqueous solution for a period of time. Poly (dopamine) was deposited on the surface of PI
films and formed PI-DOPA films. Then, PI–DOPA films were immersed into an aqueous silver nitrate solution and subjected to UV irradiation in a self-made photochemical reactor for 15 min. The PI-DOPA films deposited with silver were washed thoroughly with doubly distilled water and then dried in a vacuum oven. The distribution and size of the silver nanoparticles could be controlled by changing the reaction time. In the SEM images of films, the surface of PI-DOPA film was much rougher than that of the pristine PI film (Fig. 15.2), resulting from the formation of the distinctive poly (dopamine) layer on the PI film, which facilitated the interlocking with the reduced silver nanoparticles. The silver nanoparticles with the diameter of ~20 nm were uniformly distributed on the surface of PI-DOPA film (Fig. 15.2).

15.1.3 Vacuum Filtration

Graphene oxide (GO) and reduced graphene oxide (rGO) papers were prepared by filtration of the suspension [11]. GO colloidal suspension was prepared from graphite by the modified Hummers method [12]. The GO was reduced to rGO with the aid of hydrazine hydrate [13]. The suspension was filtrated through a PVDF filter membrane (47 mm in diameter, 220 nm pore size) by vacuum at room temperature. The paper could be easily peeled off from the filter paper. The thickness of the paper was controlled by adjusting the volume of the colloidal suspension.

The thickness of GO sheets was ~1.1 nm as measured by atomic force microscope (AFM), suggesting the formation of a single-layer 2-D nanomaterial (Fig. 15.3a), while the thickness of rGO reduced to ~1.0 nm (Fig. 15.3b). The size of GO and rGO varied from nanometers to micrometers. The resulting GO paper was of ~1.5 μm thickness, and the rGO paper was ~4.6 μm as characterized by scanning electron microscope (SEM). Interestingly, the GO paper looked lackluster while the rGO paper was lustrous (Fig. 15.3c, d).

The carbon nanotubes-deposited film or filter were fabricated by vacuum filtration, as described by the Elimelech group [14]. In a typical experiment, multi-walled carbon nanotubes (MWNTs) suspension with the concentration of 0.5 mg/mL in the...
dimethyl sulfoxide (DMSO) was sonicated for 15 min at a power output of 50 W to achieve a more uniform dispersion. Bath sonication of the MWNTs suspension was also performed for 10 s immediately prior to filter deposition to disrupt any aggregates. Deposition of MWNTs from a 5-mL solution was achieved by vacuum filtration through the PTFE membrane (5 μm pore size; Omnipore filters) to attain a loading of 0.27 mg/cm² MWNTs on the base filter. The filter was rinsed with 50 mL of ethanol followed by 50 mL of deionized water to remove residual DMSO. And the single-walled carbon nanotubes (SWNTs) filter was made from the suspension with the concentration of 0.1 mg/mL. The MWNTs-SWNTs hybrid filter was made by deposition of SWNTs on the MWNTs filter. As shown in Fig. 15.4, the surface of the MWNTs-SWNTs filter appeared similar to that of the SWNTs filter, 

Fig. 15.3 Characterization of GO and rGO nanosheets and paper [11]. (a, b) AFM images of (a) GO and (b) rGO sheets. (c) Photographs of free-standing and flexible GO (upper) and rGO (lower) (inset of (c), the photos of GO (upper) and rGO (lower) paper penetrated by white light). (d) The thickness of GO (upper) and rGO (lower) paper as measured by SEM

Fig. 15.4 FE-SEM images of the CNTs filters [14]. Aerial views of MWNT-SWNT filter (a), MWNT filter (c), and SWNT filter (e). The cross-section view of MWNT-SWNT filter (b), MWNT filter (d), and SWNT filter (f)
and both filters showed increased bundling of the SWNTs in comparison with the MWNTs filter.

### 15.1.4 Electrospinning

Schiffman et al. [15] prepared the polysulphone (PSf)/single-walled carbon nanotubes (SWNTs) composite film on the commercial filter by electrospinning technology. In the experimental process, a solution of 4 g PSf and 20 mL DMF was mixed for 24 h. Various amounts of SWNTs (0, 0.4, 20, and 40 mg, corresponding to 0, 0.1, 0.5, and 1.0 wt%, respectively) were added, followed continuous strong ultrasonication for 1 h. The PSf/DMF solution containing SWNTs was loaded into a BD Luer-Lok tip syringe (Becton, Dickinson, Franklin Lakes, NJ, USA). A Precision Glide 21-gauge needle (Becton, Dickinson) was attached to the syringe prior to securing it to an advancement pump (Harvard Apparatus, Plymouth Meeting, PA, USA). Alligator clips were used to connect the positive anode of a high-voltage supply (Gamma High Voltage Research, Ormond Beach, FL, USA) to the needle and the negative anode to a copper plate wrapped in aluminum foil. The speed of the advancement pump, separation distance between the needle and collection plate, and applied voltage were held constant at 0.8 mL/h, 7 cm, and 20 kV, respectively.

The images displayed that PSf mats electrospun with 0, 0.1, 0.5, and 1.0 wt% SWNT loadings appear white, off-white, light ash gray, and deep gray, respectively (Fig. 15.5a), and the TEM images showed that the diameter of electrospun fiber increased with the enhancement of SWNTs contain (Fig. 15.5b–e), owing to incorporation of SWNTs into the electro-spinning solution increasing its conductivity.

### 15.1.5 Magnetron Sputtering (MS)

Weng’s group [16] fabricated AgNPs/polyethylene oxide (PEO) composite film using magnetron sputtering. The fabrication process was accomplished in a bell jar vacuum chamber fed with Ar gas. Before the deposition on the p-silicon (100) wafers, the chamber was initially evacuated to a pressure below $1.3 \times 10^{-3}$ Pa, refilled with Ar gas three times, and evacuated back to $1.3 \times 10^{-3}$ Pa. In order to avoid poisoning, the sputtered target was mounted above the substrate, and the gas flowed directly to the pump after diffusing through the substrate. The silver target sputtering with 50 mm diameter, and monomer ethylene glycol dimethyl ether (EGDME) were the sources for AgNPs and PEO polymerization, respectively. DC electric power at 20 W and a suitable working pressure were employed in the MS. For the organic matrix polymerization, EGDME vapor was fed, which kept the constant flow rate by Ar (2 sccm) through the mass flow controller.
The TEM images and the selected area electron diffraction (SAED) presented the morphology of AgNPs embedded into PEO film. The pattern of discontinuous concentric rings showing in the SAED was characteristic of the silver, suggesting AgNPs in the matrix were of atomic status with preferential crystal orientation. When the working pressure was 0.2 Pa, the diameter of AgNPs with spherical shape was varied ranging from 5 to 10 nm (Fig. 15.6b), while the AgNPs were more than 20 nm when the working pressure was increased to 2.0 Pa (Fig. 15.6a), and the AgNPs shapes appeared spherical, triangular and elliptical, suggesting that the AgNPs diffused on the substrate had aggregated.

The peaks at \( 2\theta = 38.1^\circ, 44.2^\circ, 64.4^\circ, \) and \( 77.3^\circ \) that were assigned to \{111\}, \{200\}, \{220\}, and \{311\} crystalline planes of silver, respectively, also demonstrated...
that AgNPs in the composite film were still crystal (Fig. 15.6c). Based on this pattern, the size of AgNPs could be calculated using the Scherer Formula $D = \frac{K \lambda}{\beta \cos \theta}$, where $K$ depending on crystallite shape is constant (0.89), $\lambda$ is the X-ray wavelength, $\beta$ is the full width at half-max, and $\theta$ is the Bragg angle. The sizes of AgNPs were calculated as 7, 11 and 22 nm corresponding to 0.2, 1.0 and 2.0 Pa, respectively, which was in agreement with the TEM results that the AgNPs were grown with the increased working pressure.

### 15.1.6 Chemical Vapor Deposition

Chemical vapor deposition (CVD), as the most compatible approach to industrial scale production methods, could produce strongly adhesive, robust, durable, and highly active transparent thin films [17]. These film properties contrast with those produced by the coating approach that typically results in thicker films, which are less mechanically robust and often require post-coating annealing. A great many forms of CVD were developed and are frequently referenced in the literature with the different initiating means of chemical reactions and process conditions, such as atmospheric pressure CVD (APCVD), flame-assisted CVD (FACVD), thermal CVD, and so on.

Yates et al. [18] described the deposition of films of titania and copper oxide by atmospheric pressure CVD on pre-coated silica-coated barrier glass substrates. The precursor for TiO$_2$ film growth was titanium tetraisopropoxide ($7.79 \times 10^{-4}$ mol/min), transported to the reactor by N$_2$ via a bubbler. The substrate temperature for growth was set to 500°C. The CuO films were grown using an atmospheric pressure flame-assisted CVD coater with a propane/oxygen flame, previously described in detail [19]. The substrate temperature was set at 400°C. An aqueous solution of 0.5 M Cu(NO$_3$)$_2$ was nebulized into a carrier of N$_2$, through the flame and onto the substrate. The resulting films were shown to be polycrystalline. The XRD and AFM studies demonstrated that both growth of TiO$_2$ above and below CuO film was in the form of anatase, and CuO...
film deposited above the TiO₂ film was in the form of copper II oxide, and Cu I oxide when CuO film was deposited on the TiO₂ film with over 61 nm thickness. Furthermore, CuO film deposited over TiO₂ film by FACVD consisted of an island growth-type structure of packed spherical nanoparticles, with size of ~100 nm (Fig. 15.7a), which was very similar in appearance to that of a single TiO₂ layer (Fig. 15.7d), rather than that of a single CuO film (Fig. 15.7c), suggesting the CuO film deposition was very much influenced by the underlying structure, in this case the change from amorphous smooth silica to crystalline titania. However, the TiO₂ film over thick CuO film had a much more pronounced particulate structure than the other surfaces, due to the existence of Cu₂O within this TiO₂ film (Fig. 15.7b).

15.2 Antibacterial Activity of Nanomaterial-Based Films

The antibacterial activity of nanomaterial-based films, including metal oxide nanoparticles (e.g., TiO₂ and ZnO), AgNPs, graphene, and carbon nanotubes, were determined against the model bacterium *E. coli*. 

*Fig. 15.7* SEM images of (a) CuO film over TiO₂, (b) TiO₂ film over CuO, (c) CuO film and (d) TiO₂ on the glass substrates [18]
15.2.1 TiO$_2$-Based Film

TiO$_2$ nanoparticles were in the forms of anatase, brookite and rutile [20], where anatase TiO$_2$ was the most studied semiconductor after the discovery of its photocatalytic behavior [21]. So far, much attention has been focused on the photocatalysis and photo-induced hydrophilic effect mechanisms, improvement of photocatalytic activity by advancement of the microstructure, and applications including antimicrobial, and self-cleaning behaviors [22]. The biocidal activity of TiO$_2$ was first demonstrated by Matsunaga and co-workers [23]. Subsequently, a great deal of the considerable literature has shown that TiO$_2$ nanoparticles can kill cancer cells, bacteria, viruses, and algae under UV illumination [24–28], resulting in important applications in the disinfection of air, water, and surfaces. But most of these early work involved TiO$_2$ suspension and planktonic organisms. Recently, researchers had focused on the biocidal activity of the thin films of TiO$_2$ deposited on the substrate surfaces [29–32].

15.2.1.1 Comparison of the Test Methods

In order to compare the antibacterial activity of TiO$_2$ film prepared from different approaches, Yates’ group [33] firstly compared the two-test methods, BS ISO 27447:2009 and in house standard (BS EN 13697). In a typical experiment, bacterial cells were collected by centrifugation at 12,000 rpm for 5 min, and washed three times with physiological saline solution. The cells were resuspended in a 1:500 dilution of Nutrient Broth and adjusted to OD 0.1 ~ 0.2 at 600 nm in a spectrophotometer to give approximately $2 \times 10^8$ colony-forming units (CFU) per milliliter. In the BS ISO 27447:2009 method, 50 $\mu$L of cells suspension was inoculated onto each 20-mm$^2$ test sample and covered with a square of 1-mm$^2$ borosilicate glass to ensure close contact between the culture and the film. The samples were placed in 50-mm-diameter Petri dishes containing moistened filter paper to prevent drying out of the suspensions. The samples were irradiated with Blacklight Blue lamps with a maximum UV light intensity of 0.26 mW/cm$^2$. Plain borosilicate glass was used for a control experiment. Samples were removed after incubation time and immersed in 20 mL of sterile physiological saline solution, following vortexed for 60 s to resuspend the bacteria. The viability count was performed by serial dilution and plating on nutrient agar in triplicate and incubation at 37°C for 48 h. However, in the house standard method, the cells were resuspended in sterile water, and the samples were not covered with glass, but incubated in 55-mm-diameter Petri dishes kept humid by adding 2 mL sterile water under UVA lamp irradiation of 2.24 mW/cm$^2$. They found the control experiment remained viable after 24 h irradiation with only a 1-log reduction, while there was a 2-log reduction in the house standard method after 6 h irradiation. This difference could be attributed to the low concentration of Nutrient Broth in the resuspension medium in the BS ISO 27447:2009 method which meant that the bacterial cells...
were less stressed and remained viable for longer. Also, the number of the viable cells in the BS ISO 27447:2009 method was much larger that of the viable cells in the house standard method, owing to the reduced illumination levels by approximately tenfold, and the existence of oxidizable material in the resuspension medium (1/500 dilution of Nutrient Broth rather than distilled water) competing with the bacteria for reactive oxygen species (ROS).

15.2.1.2 Antibacterial Activity of TiO₂ Films

Kikuchi et al. [34] found that the survival ratio of *Escherichia coli* on the TiO₂ film under black light illumination (1.0 mW/cm²) decreased to a negligible level within 1 h (Fig. 15.8a), while the UV light only caused ~50% sterilization within 4 h. And the TiO₂ film in the dark did not affect the survival ratio, indicating that the film itself was not toxic for *E. coli*, which was also demonstrated by Sunada et al. [29]: when the initial cell concentration was 2 × 10⁵ CFU/mL (Fig. 15.8b), bacterial cells on the TiO₂ film was killed within only ~90 min under the UV light illumination (1.0 mW/cm²). And they emphasized that the survival curve did not follow a simple single exponential decay process with the increasing of illumination time, but appeared to consist of two steps, a very low rate photokilling step, followed by a higher one, which was observed regardless of the initial cell concentration in the range of 2 × 10⁵–2 × 10⁸. In the case of an initial cell concentration of 2 × 10⁵ CFU/mL, the rate constants of the first and second steps were 0.015 and 0.085 min⁻¹, respectively, which was close to those obtained in the powder systems [35, 36]. Further studies

Fig. 15.8 (a) Survival ratio of *E. coli* with and without a TiO₂ thin film under black light illumination (1.0 mW/cm²) and with a TiO₂ thin film in the dark [29]. (b) The log plot of the survival of *E. coli* cells versus illumination time. The cell suspension was incubated on TiO₂ film under UV illumination (1.0 mW/cm²). The initial cell concentrations were 2 × 10⁵ CFU/mL (■), 2 × 10⁶ CFU/mL (○), 2 × 10⁷ CFU/mL (♦), 2 × 10⁸ CFU/mL (□), respectively. Survival was also determined for cells (2 × 10⁵ CFU/ml) on the TiO₂ film in the dark (●), and on normal glass (soda-lime glass, SLG) without TiO₂ film under UV illumination (▲) (1.0 mW/cm²)
showed that various bacteria, *E. coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, etc., were killed rapidly on TiO$_2$ film under UV illumination (320–380 nm, 1.0 mW/cm$^2$) [37].

### 15.2.1.3 Antibacterial Activity of TiO$_2$-Based Composite Films

In order to overcome the disadvantages that the antibacterial activity of TiO$_2$ film was strongly weakened under the dark conditions and very low UV intensity, TiO$_2$ film deposited with the selected metal (and metal oxide) nanoparticles has been developed, such as silver and copper, which presented intrinsic antibacterial activity.

Zhang et al. [38] found AgNP-doped TiO$_2$ film exhibited much stronger antibacterial abilities toward both Gram-negative *E. coli* and Gram-positive *S. aureus* than that of pure anatase TiO$_2$ nanoparticles films. When the films were illuminated under 365 nm UV light (0.1 mW/cm$^2$), almost all the bacteria were killed by AgNP-doped TiO$_2$ films (>99.9%), while the antimicrobial value only reached 77.0% and 72.9% for *E. coli* and *S. aureus* on pure anatase TiO$_2$ nanoparticle film, respectively. More importantly, the antimicrobial activities of the AgNP-doped TiO$_2$ film were still maintained even without exposure to UV light, such that the sterilizing rate reached 99.1% and 99.4% to *E. coli* and *S. aureus*, respectively. These results indicated that AgNPs promoted the antibacterial activity of TiO$_2$. Mai et al. [4] also synthesized AgNP-doped TiO$_2$ film on titanium plates by the sol–gel process. They found AgNPs deposited on TiO$_2$ film were of metallic nature and could grow to larger ones with an increase in the annealed temperature (Fig. 15.9a–c), and that the smaller the size of AgNPs, the better was the antibacterial ability whether in the dark or under visible light (Fig. 15.9d, e).

Sunada et al. [30] prepared the copper-deposited TiO$_2$ film, and demonstrated that the resulting film could inhibit the growth of copper-resistant *E. coli* not under dark conditions, but with a very weak UV intensity of 1 µW/cm$^2$, which corresponds to the typical UV intensity of indoor light (Fig. 15.10a). However, the copper-deposited TiO$_2$ film could represent photocatalytic antibacterial activity toward the normal *E. coli* (Fig. 15.10b). Foster et al. [33] also found CuO-doped TiO$_2$ film and CuO-TiO$_2$ co-deposited film gave a 2-log reduction after 4 h in the dark, and when the incubation time increased, the antibacterial activity of CuO-doped TiO$_2$ film was higher than that of CuO-TiO$_2$ co-deposited film, resulting from the reduced availability of CuO surface on the co-deposited film. However, under the UV illumination, the antibacterial activity of both CuO-doped TiO$_2$ and co-deposited film was greatly enhanced, giving a >6-log reduction after 2 h. Furthermore, Ondok et al. [39] and Sato et al. [40] reported that the antibacterial ability of CuO-doped TiO$_2$ film was enhanced when either the content of Cu or the UV intensity increased.

Recently, Dai et al. [31] reported photocatalytic hydrogen generation using a TiO$_2$ nanoparticle/MWNTs nanocomposite under visible light irradiation, which suggests that the photocatalytic activity of the MWNTs-doped TiO$_2$ nanoparticle
Fig. 15.9 (a-c) SEM images of the AgNPs-doped TiO$_2$ films annealed at different temperature. The sizes of AgNPs in the films were 20 ~ 30 nm (a), 60 ~ 80 nm (b) and >100 nm (c). (d, e) Survival curves of *E. coli* for the films (d) in the dark and (e) at visible light irradiation. The smaller size of AgNPs have the better antibacterial ability whether in the dark or under visible light [4].

Fig. 15.10 (a) Changes in the survival of normal *E. coli* cells under the different illumination intensity. Cells ($2 \times 10^5$ CFU/mL) were incubated on the TiO$_2$ film (O) and on the copper-deposited TiO$_2$ film (□) under dark conditions, respectively. The suspension was also incubated on the copper-deposited TiO$_2$ film under UV illumination at a UV light intensity of 40 μW/cm$^2$ (■) and 1 μW/cm$^2$ (●). (b) Changes in survival of copper-resistant *E. coli* cells on the copper-deposited TiO$_2$ film under dark condition (●) and under UV illumination at light intensity of 7 μW/cm$^2$ (■) and 1 μW/cm$^2$ (▲) [30].
was excited by visible light irradiation, rather than UV irradiation. Akhavan et al. [41] and Oh et al. [42] have shown that the MWNTs/TiO2 nanocomposite could inactivate bacteria under the visible light irradiation and in the dark. Further, Akhavan et al. [32] tested the antibacterial property of MWNTs-doped TiO2 film by varying the content of MWNTs and the post-annealing temperature. They found that the antibacterial activity of the MWNT-doped TiO2 films in the dark gradually increased by increasing the MWNTs content of the films, independent from the post-annealing temperature (Fig. 15.18a). Especially, when the MWNTs contain reached 20 wt%, the MWNTs-doped TiO2 films annealed at 450°C showing an ability of complete inactivation of the bacteria under the visible light irradiation for 1 h, while the corresponding film annealed at 100°C could kill 93% of the bacteria under the same conditions.

15.2.1.4 Durability and Regenerate Ability of TiO2 Films

Book and co-workers [43] demonstrated the durability and regenerate ability of TiO2 film prepared by CVD. TiO2 films were repeatedly cycled through the biocidal test procedure followed with a cleaning process (film was sonicated in methanol and then chloroform for 30 min), and then characterized the film photoactivity by the stearic acid test. They found no measurable reduction in maintained photoactivity, within the accuracy of the test, over three test cycles (Fig. 15.11b).

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**Fig. 15.11** (a) SEM image of TiO2 over AgNPs films. (b) An example sample showing the retention of photoactivity after bioactivity testing. (c) Photoactivity after bio-contamination and (d) photoactivity after UV “self-regeneration” [43]
Additionally, it was known that TiO$_2$ film was mechanically durable, owing to the TiO$_2$ was hard and scratch resistant and had the longest term stability. The TiO$_2$ surface can decompose organic contamination with the aid of UV light, suggesting the application of TiO$_2$ photocatalysis to novel “self-cleaning” techniques, which was first demonstrated by Watanabe et al. in 1992 [44]. And water could penetrate the molecular-level space between the stain and the superhydrophilic TiO$_2$ surface, so the surface was maintained clean with the supply of water current even though the photons excited by UV light may be insufficient to decompose the adsorbed stain [45]. After the biocidal test procedure, the TiO$_2$ film was visibly contaminated with dead bacteria residues, leading to significant reduction of photocatalytic activity (Fig. 15.11c). However, after the film contaminated with dead bacteria residues was treated with an additional UV irradiation, the film recovered a significant percentage of the original activity (Fig. 15.11d).

### 15.2.2 ZnO-Based Films

Owing to low cost, easy availability and unique chemical and physical properties, ZnO nanoparticles has sparked much interest. Jones et al. [46] reported that ZnO nanoparticles presented higher antibacterial activity on *S. aureus* than other metal oxide nanoparticles. Padmawathy et al. [46] demonstrated that nano-ZnO showed enhanced antibacterial activity as compared with bulk ZnO. And Zhang et al. [47] reported that the antibacterial activity of ZnO nanoparticles increased with decreasing particle size, and the dispersants (Polyethylene Glycol and Polyvinylpyrolidone) did not much affect the antibacterial activity of ZnO nanoparticles but enhanced the stability of the suspensions. Thus, Bajpai et al. [48] prepared ZnO/chitosan film, and revealed that the film showed excellent antibacterial action against *E. coli*. And Chandramouleeswaran et al. [48] demonstrated that nano-ZnO/polypropylene film could kill almost all *Staphylococcus aureus* and *Klebsiella pneumoniae* with just nano-ZnO filler at a 3% level of loading. Shalumon et al. [49] demonstrated that sodium alginate/poly(vinyl alcohol)/nano-ZnO composite nanofiber mats could suppress the growth of *Staphylococcus aureus* and *Escherichia coli*.

### 15.2.3 AgNPs-Based Films

The antimicrobial properties of silver were well known to the ancient Egyptians and Greeks. Since then, silver has been used in different fields in medicine and surface coating for many years [50], due to a strong cytotoxic effect toward a broad range of microorganisms and remarkably low human toxicity compared to other heavy metal ions. Silver nanoparticles (AgNPs) also show efficient antimicrobial properties, because of their extremely large surface area which provides better contact with
microorganisms. Recently, not only silver ions or a silver nanoparticle colloid but also all kinds of silver-based films have attracted more and more attention.

Akhavan et al. [51] synthesized AgNPs film on the SiO$_2$ thin film, and found that the AgNPs film presented strong antibacterial activities against *E. coli* and *S. aureus* bacteria with relative rates of reduction of the viable bacteria of 1.05 and 0.73 h$^{-1}$ for initial concentration of $\sim 10^5$ CFU/mL, respectively, and the difference was attributed to amount of peptidoglycan in the cell wall structure. The antibacterial activity of the AgNPs films was dependent on the AgNPs size corresponding to the surface-to-volume ratio. The smaller AgNPs with larger surface area could lead to a much greater bactericidal effect [52].

However, AgNPs were not stable and readily aggregated, and AgNP oxidation was accelerated by illumination with white lamps in air [53], resulting in the reduction of antibacterial activity. Therefore, the AgNP-based composite films, such as AgNP/TiO$_2$ film [54], AgNP/chitosan film [55–57], AgNP/polyethylene oxide film [16], AgNP/hyaluronan/poly (dimethylallylammonium chloride) film [58], AgNP/sodium alginate film [59], AgNP/polyvinyl alcohol [60], AgNP/polyester film [61], AgNP/poly (ethylenimine) film [7], AgNP/polyvinyl sulphonate film [62], AgNP/poly (vinyl alcohol/poly (L-lactic acid) film [63], and AgNP/N-(2-aminoethyl)-3-aminopropyl-trimethoxysilane (DIAMO) film [64] were considered to overcome those challenges. Akhavan et al. [65] found the antibacterial activity of TiO$_2$-capped silver nanorods film in the dark was stronger than that of TiO$_2$-capped AgNPs film, with 2.34 h$^{-1}$ for the relative rate of reduction of the number of viable bacteria. Vimala et al. [66] successfully fabricated the AgNP/chitosan film by a three-step process: silver ion-poly (ethylene glycol) matrix preparation, addition of chitosan matrix, and removal of poly (ethylene glycol) from the film matrix. The AgNP/chitosan film can inhibit the growth of *E. coli*, *Bacillus*, and *K. pneumoniae*, and especially, after 350 min of incubation, AgNPs-chitosan film can killed $\sim$75% of *E. coli*.

### 15.2.4 CNTs-Based Films

Carbon nanotubes are pseudo-one-dimensional carbon allotropes of high aspect ratio, high surface area, and excellent material properties, such as ultimate electrical and thermal conductivities and mechanical strength, which offer a wide range of opportunities and application potential in biology and also antibacterial nonmaterial [67]. Narayan et al. [68] showed that nanotube films formed via high temperature laser ablation of graphite on silicon completely inhibited bacterial colony formation. However, the nanotube structure was not well controlled and the process may not be amenable to many biomedical materials. Kang et al. [69], Rodrigues et al. [70] and Brady-Estévez et al. [71] tested the antibacterial activity of SWNTs filter against *E. coli*, showing inactivation of $\sim$80% of *E. coli* after only 20 min incubation (Fig. 15.12) [71]. And then Kang et al. [69, 72] has revealed that the SWNTs filter presented higher antimicrobial activity than a MWNTs-coated filter, and the
SWNT-coated filter could inactivate >60% of microorganisms in river water and wastewater treatment plant samples, while natural organic matter did not influence its antimicrobial activity [72]. Brady-Estevez et al. [14] prepared a novel SWNTs-MWNTs hybrid filter which was composed of a thin SWNTs layer on top of a thicker MWNTs layer supported by the PTFE membrane, and found that the hybrid filter not only exhibited high log removal of several model viruses (MS2, PRD1, T4) by depth filtration but also provided high levels of inactivation of model bacteria (E. coli K12 and Staphylococcus epidermidis), as well as microbes from river water and treated wastewater effluent.

They found the physicochemical properties (e.g., diameter, length, aspect ratio, sample purity, structural defects) determined the antimicrobial activity of MWNTs-coated filter, and functionalized [sonication in a mixture of H2SO4 and HNO3 (3:1 v/v) for 1 h] and short MWNTs-coated filters represented excellent antibacterial activity, possibly due to increased density of the open tube ends [73]. However, Yang et al. [74] considered that longer SWNTs filters exhibited stronger antibacterial activity. Vecitis et al. [75] demonstrated for the first time that SWNTs electronic structure was a key factor regulating SWNTs antibacterial activity, and found that antibacterial activity of the high percent metallic (>95%) SWNTs filter was higher than that of the low percent metallic (<5%) SWNTs one, owing to the high percent metallic SWNTs-induced oxidative stress after SWNTs–bacteria contact and physical perturbation of the cell membrane.

In order to improve the antibacterial property of CNTs filter, large amounts of CNTs-based composite filters were introduced. Simmons et al. [76] prepared a flexible composite film by depositing SWNTs coated with polyvinylpyrrolidone-iodine (PVPI) in water, and found that the PVPI-coated SWNTs film could slowly release antiseptic iodine, resulting in the effective antibacterial property over 48 h incubation. Aslan et al. [77] found that E. coli and S. epidermidis viability and metabolic activity were significantly diminished on the SWNTs/polymer poly(lactic-co-glycolic acid)
(PLGA) film, and were correlated with SWNTs length and concentration (<2 wt%). Schiffman et al. [15] observed that the loss of viability of *E. coli* on the electrospun polysulphone/SWNTs mats was directly correlated to increased SWNTs incorporation within the mat, ranging from 18% for 0.1 wt% SWNTs to 76% for 1.0 wt% SWNTs, and the antimicrobial action of the polysulphone/SWNTs mats occurred after a short contact time of 15 min or less. Pangule et al. [78] incorporated conjugates of MWNTs with lysostaphin (Fig. 15.13a), a cell wall degrading enzyme, into films to impart bactericidal properties against methicillin-resistant *S. aureus* (MRSA) and *S. epidermidis*, and found that these enzyme–MWNTs films were highly efficient in killing MRSA (>99% within 2 h) without release of the enzyme into solution (Fig. 15.13b), and these films were reusable and stable under dry storage conditions for a month (Fig. 15.13c). Zhou and Qi [79] synthesized a novel epsilon–polylysine–MWNTs nanocomposite by covalent attachment of epsilon-polylysine on MWNTs with hexamethylene diisocyanate as the coupling agent,

![Scheme of antimicrobial nanocomposite films containing lysostaphin-MWNTs.](image)

**Fig. 15.13** (a) Scheme of antimicrobial nanocomposite films containing lysostaphin-MWNTs. (b) Comparison of bactericidal effect of Lst-MWNTs film (■) and Lst-PEG-MWNT film (▲) with the native enzyme (♦). These enzyme-MWNTs films were highly efficient in killing MRSA without release of the enzyme into solution. (c) Operational (●) and storage stability (■) of films containing 4% w/w Lst in the form of Lst-PEG-MWNT. The films were stored in dry conditions and at room temperature in between the two use cycles [78]
and found that the epilson–polylysine–MWNTs film showed improved antibacterial activities and excellent anti-adhesive efficacy against *P. aeruginosa* and *S. aureus*.

Additionally, owing to outstanding electron transmitting property of CNTs, the antibacterial activity of CNTs films was enhanced by the aid of applied potential. Vecitis et al. [80] prepared an electrochemical MWNTs microfilter by depositing MWNTs on the PTFE membrane, and demonstrated that the MWNTs filter was effective for complete removal of bacteria by sieving and multilog removal of viruses by depth filtration in the absence of electrolysis, while concomitant electrolysis during filtration resulted in significantly increased inactivation of influent bacteria and viruses; especially, application of 2 and 3 V for 30-s post-filtration inactivated >75% of the sieved bacteria and >99.6% of the adsorbed viruses, leading to the number of bacteria and viruses in the effluent reaching below the limit of detection.

### 15.2.5 Graphene-Based Films

Graphene consisted of a monolayer of carbon atoms which were tightly packed into a two-dimensional crystal. Since the seminal work of Geim and coworkers on free-standing graphene in 2004 [81], many potential applications of graphene were actively pursued owing to its outstanding mechanical stiffness and electronic transport property [82, 83], such as nano-electronic devices [84], sensors [85], solar cells [86], and nanocomposite materials [82].

Hu et al. [11] demonstrated that the two water-dispersible graphene derivatives, graphene oxide (GO) and reduced graphene oxide (rGO) nanosheets, could effectively suppress the growth of *E. coli* cells, and the free-standing GO and rGO papers also presented antibacterial activity. And Akhavan et al. [87] further demonstrated that GO and rGO nanowalls could kill Gram-positive *S. aureus* bacteria. Park et al. [88] fabricated the Tween/rGO paper by simple filtration of a homogeneous aqueous colloidal suspension of a Tween/rGO hybrid, and found that the tween/rGO paper could inhibit nonspecific binding of Gram-positive bacteria *Bacillus cereus*, while rGO paper without tween showed nonspecific bacteria binding (Fig. 15.14).

### 15.3 Mechanism of Nanomaterial-Based Films Antibacterial Activity

#### 15.3.1 TiO$_2$-Based Films

As we know, the antibacterial activity of TiO$_2$ film was attributed to its photocatalysis property. In order to reveal the important molecules directly interacting with bacterial cells, Kikichi et al. [34] designed the membrane-separated
system to fence out TiO$_2$ film and *E. coli* suspension by the PTFE membrane (50 μm thickness, 0.4 μm pore size). They found the survival ratio of bacteria on the film surface improved with the increase in both mannitol concentration and pH value which could suppress the activity of radical molecules (•OH and •O$_2$⁻), and that the existence of catalase in the suspension could enhance the bacterial survival ratio, suggesting the formation of radical molecules and H$_2$O$_2$ in the suspension under the UV illumination. So, it was clear that various reactive species (e.g., •OH, HO$_2$•, H$_2$O$_2$) were produced by UV illumination of TiO$_2$ in the presence of water and air by the following reactions [89, 90]

\[
\text{TiO}_2 + h\nu \rightarrow e^- + h^+ \quad (15.1)
\]

\text{<Reduction reaction>}

\[
\begin{align*}
O_2 + e^- &\rightarrow O_2^- \\
O_2^- + H^+ &\rightarrow \text{HO}_2^* \\
\text{HO}_2^* + \text{HO}_2^* &\rightarrow \text{H}_2\text{O}_2 + O_2 \\
\text{H}_2\text{O}_2 + e^- &\rightarrow \text{OH}^- + \text{•OH} \quad (15.2)
\end{align*}
\]
<Oxidation reaction>

\[
\begin{align*}
H_2O + h^+ \rightarrow & \cdot OH + H^+ \\
\cdot OH + \cdot OH \rightarrow & H_2O_2 \\
H_2O_2 + 2h^+ \rightarrow & 2H^+ + O_2
\end{align*}
\] (15.3)

These reactive oxygen species (ROS) can decompose organic compounds and extinguish cellular activity.

Sunada et al. [29] studied the photokilling process of *E. coli* on the TiO₂ film by means of AFM, which suggested that bacterial cells decomposed from the outside of the cell, resulting from the TiO₂ film photocatalysis (Fig. 15.15a–c). Additionally, Kühn et al. [91] observed that the killing rates of bacteria were dependent on the thickness and structure of cell walls. Based on these observations, they found that the photokilling of bacteria on the illuminated TiO₂ surface could be divided into three stages (Fig. 15.15d). First, disordering of the outer membrane of bacterial cells by reactive species (•OH, •O₂⁻, H₂O₂). The outer membranes of *E. coli* cells were decomposed partially by the reactive species produced by the TiO₂ photocatalyst, while the bacteria cell viability was not lost very efficiently. Second, disordering of the inner membrane (the cytoplasmic membrane) and killing of the cell. Owing to the change of the permeability to reactive species when the partial outer membrane was decomposed, reactive species easily reached and attacked the inner membrane,
leading to the peroxidation of the membrane lipid. The structural and functional disordering of the cytoplasmic membrane due to lipid peroxidation led to the loss of cell viability and cell death. And third, decomposition of the dead cell. If the illumination continued for a sufficiently long time, the dead cells were found to be decomposed completely.

As to the CuO-doped TiO2 film, Sunada et al. [30] presumed that the valence state of the copper played the key role in the bactericidal process both in the dark and under the very weak UV illumination. It has been reported that the copper ions and metallic copper could be transformed into each other by the following redox reaction by the help of photo-generated electrons and holes [92–98].

\[
\begin{align*}
\text{<Reduction reaction>} & \\
\text{Cu}^{2+} + e^- & \rightarrow \text{Cu}^+ \\
\text{Cu}^+ + e^- & \rightarrow \text{Cu}
\end{align*}
\]

(15.4)

\[
\begin{align*}
\text{<Oxidation reaction>} & \\
\text{Cu} + h^+ & \rightarrow \text{Cu}^+ \\
\text{Cu}^+ + h^+ & \rightarrow \text{Cu}^{2+}
\end{align*}
\]

(15.5)

Litter et al. [99] and Ciesła et al. [100] reported that the photocatalytic activity of TiO2 was enhanced through converting photo-generated H2O2 into more reactive \( \cdot \text{OH} \) by the following copper-mediated Fenton-type reactions.

\[
\begin{align*}
\text{H}_2\text{O}_2 + \text{Cu}^+ & \rightarrow \cdot \text{OH} + \text{OH}^- + \text{Cu}^{2+} \\
\text{Cu}^{2+} + e^- & \rightarrow \text{Cu}^+ \\
\text{or} \\
\text{Cu}^{2+} + \text{O}_2^- & \rightarrow \text{Cu}^+ + \text{O}_2
\end{align*}
\]

(15.6)

Sato et al. [40] surmised a possible mechanism of the enhanced antibacterial activity of CuO-doped TiO2 film, which was associated with photocatalysis under the weak UV illumination (Fig. 15.16). Through a series of photoreactions as expressed above, reactive oxygen species (ROS) such as \( \cdot \text{OH}, \cdot \text{O}_2^- \) and H2O2, were generated by TiO2 photo-excitation on the CuO-doped TiO2 film surface. Because H2O2 was more stable than \( \cdot \text{OH} \) and \( \cdot \text{O}_2^- \), it could diffuse from the CuO-doped TiO2 film surface into the suspension. As shown in Fig. 15.16a, a small amount of copper ion could leach out of the CuO-doped TiO2 solid phase into the suspension and was reduced into Cu + by receiving electrons from photo-excited TiO2 nanoparticles. Then, the free Cu + reacted with H2O2 to produce \( \cdot \text{OH} \) via Fenton-type reactions, contributing to the deactivation of microbial cells in the suspension. On the other hand, the following reactions are supposed to occur in solid phase (CuO-doped TiO2 film) (Fig. 15.16b). Cu2+ could be reduced into Cu + by electron from photo-excited TiO2, and in turn Cu + converted H2O2 into...
-OH, while being oxidized into Cu\(^{2+}\) again, leading to much more deactivated bacterial cells. However, Sunada et al. [30] proposed that copper ions could penetrate the damaged membrane into cytoplasm, resulting in a direct disturbance in intracellular metabolic systems, and thus the bactericidal process for the bacterial cells on the CuO-doped TiO\(_2\) film under weak UV illumination consisted of two steps (Fig. 15.17). First, the outer membrane was attacked by the reactive oxygen species produced by TiO\(_2\) photocatalysis and the transformation between Cu\(^{2+}\) and Cu\(^+\) (Fig. 15.17b, c). And then copper ions (maybe and Cu\(^+\)) were effectively taken into the cytoplasmic membrane (Fig. 15.17c, e). In this case, the photocatalytic reaction mainly played a critical role in assisting the intrusion of copper ions into the cells.

Therefore, the metal nanoparticles may play three roles in killing bacteria: (1) it could prevent photo-generated electrons and holes from surface recombination by trapping of photo-generated electrons with positive metal ions; (2) it increased the yield of hydroxyl radical through the Fenton-type process by reaction with photo-generated H\(_2\)O\(_2\), and (3) it diffused into the cytoplasmic membrane of the bacteria and accelerated the lethal effect after the outer membrane of the bacteria was destroyed by oxidizing oxygen species. In addition, the metal ions can kill bacteria directly, which could explain the antibacterial activity of CuO-doped TiO\(_2\) film in the dark.

On the other hand, Akhavan et al. [42] considered that the improved antibacterial property of the MWNTs-doped TiO\(_2\) film could be assigned to the formation of Ti–C and Ti–O–C carbonaceous bonds at 450° C, which was confirmed by the their XPS results. So a possible mechanism of the improvement in the photo-inactivation was proposed (Fig. 15.18b). First, the electrons generated by the photo-excited MWNTs were transmitted to the conduction band of the TiO\(_2\) through the Ti–C bonds, leading to the formation of the positively charged MWNTs, which could capture electrons from the valence band of TiO\(_2\) to generate the holes in the TiO\(_2\) [101, 102]. And the electrons and holes induced the generation of ROS by a series

![Fig. 15.16 Schematic illustration showing possible mechanism of deactivation on CuO-doped TiO\(_2\) film in the liquid phase (a) and on the solid surface (b) [40]](image.png)
Fig. 15.17  Schematic illustration of the bactericidal process for the copper-resistant *E. coli* cell on the normal TiO$_2$ film and on the CuO-doped TiO$_2$ film: (a) illustration of *E. coli* cell, (b–e) enlarged illustration of cell envelope parts [30]

Fig. 15.18  (a) Percentage of survival ratio of *E. coli* bacteria on the surface of the MWNTs-doped TiO$_2$ film annealed at 100°C and 450°C with various MWNTs contents in the dark and under visible light irradiation for 1 h. (b) Schematic illustration of photocatalytic mechanism of the MWNTs-doped TiO$_2$ film under visible light irradiation [42]
of reactions as above described. And second, the formation of the heterojunction between TiO$_2$ and MWNTs resulted in giving rise to a charge space near the junction to equalize Fermi levels, ranging from several tens to hundreds of nanometres. Not only was the band gap energy within the MWNTs-TiO$_2$ heterojunction smaller than the band gap energy of the TiO$_2$ located out of the junction but also a driving force originated from interior electric field of the charge space could separate the photo-generated pairs which resulted in reduction of the recombination rate of the pairs. Additionally, the natural bactericidal activity of MWNTs also contributed to this property in the dark.

### 15.3.2 ZnO-Based Films

Premanathan et al. [103] suggested that ZnO nanoparticles killed HL 60 cells by generation of ROS and induction of apoptosis. The mechanism of antibacterial activity of ZnO nanoparticles was not well understood although the ZnO nanoparticles could effectively inhibit both Gram-positive and Gram-negative bacteria [104–107].

Antibacterial activity of ZnO nanoparticles may be attributed to several mechanisms. First, by induction of oxidative stress which led to interaction with proteins, DNA, and lipids causing death [108–110]. In 1996, Sawai et al. [107] discovered that H$_2$O$_2$ was produced in ZnO slurry and the concentration of H$_2$O$_2$ was linearly proportional to the ZnO particles, which confirmed by Yamamoto et al. [111]. It is known that ZnO possesses photocatalytic activity under the UV light [112]. However, most of the antibacterial tests were done under the dark conditions, so it was still not clear how the ROS species were produced and how to improve the active oxygen production in the dark. Second, by membrane destruction due to accumulation of ZnO nanoparticles in the bacterial membrane and also their cellular internalization [113]. Zhang et al. [47] showed that the ZnO nanoparticles damages the membrane of the bacterial cells by the aid of TEM studies, and the electrochemical measurements via a model DOPC monolayer further confirmed the direct interaction between ZnO nanoparticles and the bacterial membrane. And third, by the release of Zn ions that may be responsible for antimicrobial activity by contracting with the membrane of microorganisms [114]. However, the toxicity of ZnO nanoparticles was not directly related to their entering into the cell, rather their intimate contact onto the cell causes changes in the microenvironment in the vicinity of the organism–particle contact area to either increase metal solubilization or to generate ROS [115], which may ultimately damage the cell membrane [116].

### 15.3.3 AgNPs-Based Films

Although AgNP-based films represented excellent antibacterial activity, the antibacterial mechanism was not completely understood. Generally, it was clear
that the antimicrobial property of silver was related to the amount of silver and the rate of silver released. Silver in its metallic state was inert, but it reacted with moisture, and become ionized. The ionized silver was highly reactive. Silver ions interacted with thiol groups of membrane-bound enzymes and proteins, resulting in membrane structure and permeability changes [117–119]. After penetrating through the cell membrane, silver ions could uncouple the respiratory chain from oxidative phosphorylation [120], and bind to DNA and RNA by denaturing and inhibiting bacterial replication [121].

The antibacterial property of AgNPs-based films was attributed to silver ions generation and unique nanostructure of AgNPs. On the one hand, Akhavan et al. [51] demonstrated that silver ions were released over long periods from the film surface, even from TiO2-capped AuNPs and silver nanorods films (Fig. 15.19) [65]. Agarwal et al. [122] also found that localization of AgNPs on the AgNPs/poly (allylamine hydrochloride)/poly (acrylic acid) film generated the concentrations of silver ions required for antibacterial activity at the surface, without requiring the high loading of silver AgNPs. On the other hand, free AgNPs released from films could directly interact with the microorganism by disrupting/penetrating the cell envelope, and generating reactive oxygen species (ROS) [108, 109] that caused deadly damage. Moreover, free AgNPs preferentially attacked the respiratory chain, cell division finally leading to cell death [123, 124]. So, compared to silver ions, the effective concentration of AgNPs was \(~10^3\)-fold lower, being at the nanomolar level [125]. In the AgNPs-based composite film, the improvement of antibacterial activity also partly arose from the action of the other components, such as AgNPs/TiO2 films [126] and AgNPs/ZnO film [127].

Fig. 15.19 (a) CFU of E. coli cultured for various periods in the medium containing different films. (b) The silver ion release curves of the TiO2-capped AgNPa and silver nanorods films. (a) TiO2-capped Ag film in the dark. (b) TiO2-capped AgNPs film in the dark. (c) TiO2-capped silver nanorods film in the dark. (d) Silver nanorods film in the dark. In the inset of (a): TiO2-capped silver nanorods film in the dark (c) and under UV irradiation (d); (e) blank sample [65]
15.3.4 CNTs-Based Films

Despite agreement about the potential toxicity of CNTs on mammalian cells, the mechanism of CNTs toxicity is still elusive. Previous studies have proposed three hypothesized mechanisms: oxidative stress [128, 129], metal toxicity [130, 131], and physical piercing causing rupture [68, 132]. Where the generation of reactive oxygen species (ROS) and oxidative stress are the most developed paradigms for the mechanism of CNTs cytotoxicity. However, how do CNTs exert their antimicrobial activity? Two possible mechanisms have been proposed, i.e. mechanical disruption, where nanotubes act to physically pierce or otherwise perturb the bacterial membrane, and oxidative stress, where the high reductive potential of nanoscale carbon induced the generation of ROS to damage cell membranes and to disturb the metabolic pathway.

Kang et al. [69] and Brady-Estévez et al. [71] studied the scanning electron microscopic (SEM) images of CNTs-treated *E. coli*, and found that the treated *E. coli* cells became flattened, and lost their cellular integrity, suggesting irreversible cell membrane damage and cell death (Fig. 15.20), which confirmed that the antibacterial activity of CNTs was related to the surface characteristics of the bacteria [14]. The toxicity of CNTs against Gram-positive bacteria was smaller than that of CNTs against Gram-negative bacteria, owing to the thicker peptidoglycan in Gram-positive bacterial cell walls [133]. In 2008, Kang et al. [134] further found that the cytoplasmic nucleic acids were presented in the culture medium by DNA and RNA assays, and that genes related to the cell envelope integrity, including fatty acid biosynthesis, Tol/Pal system [135–137], and PhoPQ two-component system [138], were up-regulated in the SWNTs-treated *E. coli*, confirming the cell membrane damage. However, another kind of gene related to oxidative stress (e.g. *soxRS*, *oxyR*) was up-regulated in the DNA micro-array analysis, suggesting that oxidative stress was also responsible for the antibacterial property of CNTs.

Therefore, a three-step interaction process between CNTs and bacteria was proposed. First, by initial CNTs–bacteria contact. The bacteria were deposited

Fig. 15.20  SEM images of *E. coli* incubated for 60 min without (a) and with (b) SWNTs. The cell membrane of treated *E. coli* was damaged, leading to cell death [69]
onto CNTs resulting in direct bacteria–CNTs contact which could be mediated by the exposed CNTs surface area, bacteria concentration, and solution composition. Second, by perturbation of the cell membrane. The mechanism of this step could be clarified according to the toxicity of various classes of hydrocarbons against aquatic microorganisms [139]. The toxicity of hydrocarbons involved two parts: the nonspecific and specific toxicity. The nonspecific toxicity was the disruption of the cell membrane, and dependent on the hydrophobicity of hydrocarbon. And the specific toxicity was described as how the hydrocarbons affected membrane proton transport, disrupted specific proteins, or chemically oxidized biomolecules (i.e. proteins, lipids, and DNA), which was attributed to the electrophilicity of hydrocarbon [140, 141]. Furthermore, Kang et al. [73] demonstrated that the perturbation of cell membrane was related to diameter and hydrophobicity of CNTs, the intensity of the open tube ends. And third, by bacterial oxidation. The cell death resulted from the ROS oxidation and regulation of cellular metabolic pathway (Fig. 15.21). The ROS were generated by the cell membrane damage and the interactions between CNTs and biomolecules (e.g., GSH [75]).

Vecitis et al. [80] proposed two primary mechanisms of electrochemical inactivation of *E. coli* and MS2: the direct oxidation of pathogen in contact with the MWNTs anode, and the indirect oxidation of pathogen via anodic production of an aqueous oxidant (e.g., Cl2−, HO•, or SO4−2). During the process, MWNTs provided positive holes (h+). The oxidation reaction of pathogen with MWNTs and anodic one-electron oxidant resulted in the cell membrane damage and cell death, further confirmed by the SEM images of different potential-treated *E. coli*.

![Fig. 15.21 Schematic summary of *E. coli* K12 gene expression stress responses under exposure to SWNTs and MWNTs, suggesting the mechanism of CNTs-induced antibacterial activity [73]](image)
15.3.5 Graphene-Based Films

The antibacterial mechanism of graphene-based paper was attributed to graphene-induced cell membrane damage after interactions between graphene derivatives and bacterial cells. Akhavan et al. [87] reported that concentrations of RNA in the solutions of the bacteria exposed to the both GO nano-walls and rGO nano-walls were meaningfully higher than that of the control sample (Fig. 15.22a, b), suggesting the bacteria membrane damage. And the SEM images of *E. coli* attached on the surfaces of GO and rGO papers showed that treated *E. coli* cells on the paper lost the integrity of membranes (Fig. 15.22c, d) [11], further confirming this hypothesis. Additionally, in the tween/rGO composite paper, the tween-20 could prevent bacteria from adhering on the paper owing to its amphiphilic property [88].

15.4 Toxicity of Nanomaterial-Based Films

With the development of nanotechnology, artificial nanomaterials meeting different requirements were designed and applied. As a result, the biological effects of these artificial nanomaterials on humans and the environment became more and more

![Fig. 15.22](image_url) (a, b) Cytotoxicity of GO nanowalls and rGO nanowalls to *E. coli* (a) and *S. aureus* (b), and concentrations of RNA in the PBS of the bacteria exposed to the nanowalls [87]. (c, d) Photographs of *E. coli* growth on GO (c) and rGO (d) paper (overnight incubation at 37°C) [11]
important. Owing to differences in test methods, the biological effect of the same nanomaterial was not always in agreement. However, it was known that high concentration of nanomaterials was toxic to mammalian cells.

Lagopati et al. [26] reported that TiO$_2$ nanoparticles could kill the mammalian cells via reactive oxygen species generated by photocatalysis, while Kommireddy et al. [142] revealed that TiO$_2$ thin film could speed the spread of mouse mesenchymal stem cells owing to the rough surface. The published literature shows that ZnO nanoparticles have been applied to drug delivery and cosmetics with non-toxicity [143], while Hanley et al. [144] demonstrated that ZnO nanoparticles could kill cancerous T cells owing to the generation of ROS. Huang et al. [145] also reported the cytotoxicity of ZnO nanoparticles with the size of 20 nm against human bronchial epithelial cells (BEAS-2B) was concentration- and time-dependent, resulting from elevating oxidative stress, disturbing calcium homeostasis and causing membrane damage. However, ZnO nanoparticles were considered and generally recognized as safe (GRAS) material by FDA [146].

Although silver could effectively suppress the growth of microorganisms, it also led to dose-related toxicity in tissue. It has been reported that silver ions accumulated in epithelial cells, macrophages, fibroblasts, and connective tissue [147, 148] and caused tissue toxicity and impaired wound healing [149, 150]. In vitro studies have also demonstrated that the concentrations of silver released from AgNPs-based film could be cytotoxic to mammalian cells involved in wound healing, including fibroblasts [151, 152], keratinocytes [151], and lymphocytes [153]. However, it was found that human osteoblast attached and grew well on the surface containing AgNPs [154, 155]. Agarwal et al. [122] employed molecularly thin polymeric films [poly (allylamine hydrochloride) and poly (acrylic acid)] prepared by layer-by-layer deposition to localize AgNPs on surfaces, and found that the resulting composite film could release silver ions, leading to antibacterial activity without cytotoxicity. Also, Zan et al. [63] considered that the high resistance of AgNPs/poly (vinyl alcohol)/ploy (L-lactic acid) film to HeLa cells is not due to the embedded AgNPs, but to its high water content, hydrophilicity, and low interfacial tension between the hydrogel surface and the surrounding fluids.

Generally speaking, carbon-based nanomaterials were regarded as “safe” since the carbon element was inherently compatible with living systems. However, the high concentrations of CNTs and graphene derivatives were cytotoxic to the mammalian cells [132, 133], and the cytotoxicity could be mitigated by chemical modification [156–158]. Agarwal et al. [159] demonstrated that SWNTs thin film inhibits the proliferation, viability, and neurogenesis of PC 12 cells, and the proliferation of osteoblasts. The CNTs films can improve neural signal transfer [160]. The tendency of graphene oxide cytotoxicity was similar to that of CNTs cytotoxicity [161]. Agarwal et al. [159] and Chen et al. [162] showed that graphene-based paper displayed good biocompatibility against neuroendocrine PC 12 cells, osteoblast, and mouse fibroblast cell line L-929. Park et al. [88] also demonstrated that tween/rGO paper showed no cytotoxicity against African green monkey kidney cells, embryonic bovine cells, and Crandell Rees feline kidney cells.
15.5 Applications of Nanomaterial-Based Films

Despite the considerable concerns on public health and food safety, antibacterial materials have become more and more important in everyday use. However, traditional antibacterial materials have raised significant concerns on antibiotic resistance, environmental pollution, relatively complex processing and high cost [163, 164]. Owing to their excellent antibacterial property, nanomaterials-based films can inactivate bacteria attached on the surface, and thus nanomaterial-based films have been extensively applied to self-sterilizing surfaces of materials in public locations, food storage and clinical facilities, such as hospitals, elderly care facilities, wound care dressings, and orthopedic implants, where it was critical to control the surface and airborne bacteria.

Antimicrobial food packaging materials have been used to extend the lag phase and reduce the growth rate of microorganisms in order to extend shelf life and to maintain product quality and safety. Emamifar et al. [95] used AgNPs/LDPE and ZnO/LDPE films to store orange juice at 4 °C for 112 days, and found that the films could significantly suppress the growth of Lactobacillus plantarum in the orange juice, suggesting promising applications of nanomaterial-based film in food packaging.

Fujishima et al. [37] developed antibacterial tiles by covering ordinary tiles with TiO₂-Cu composite film, and tested such tiles on the floor and walls of the hospital operating room. The results showed that the bacterial counts decreased to negligible levels in a period of 1 h, and surprisingly, the bacterial counts in the surrounding air also significantly decreased, leading to commercial applications of such tiles in hospitals, hotels, and restaurants, among others [22]. Ohko et al. [121] fabricated TiO₂ film-coated silicone catheters, with repeated bending and resistibility to scratching. Further clinical studies showed TiO₂ film-coated silicone catheters presented better antibacterial activity compared to conventional catheters, suggesting the promising clinical application as an alternative to conventional catheters [165].

15.6 Conclusions

Recently, microorganism safety has attracted increasing attention. Particularly, the Severe Acute Respiratory Syndromes (SARS) virus, H1N1 flu virus and super-bacteria are seriously endangering people’s health. The development of new nanomaterial-based antibacterial paper may provide a unique solution. In this chapter, we have summarized the preparation, antibacterial activity, and mechanisms, as well as potential applications, of nanomaterial-based paper. Although there still exist many challenges, such as the high cost, complex synthesis process, and environmental impact, we have witnessed significant advances toward the design and fabrication of novel antibacterial materials that may eventually find real-world applications.
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