



Antimicrobial Potential of Titanium Dioxide Nanoparticles in Urinary Tract Infections: An Experimental Study on the Growth Inhibitory Activity and Biofilm Inhibition

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Abstract

Background: Microorganisms cause many diseases for the human body such as urinary tract infection and, therefore, it is highly important to eliminate and control them. Bacterial resistance to different types of antibiotics was increased and it is necessary to find alternative agents to eliminate these microbes.

Methods: This study aimed to evaluate the antimicrobial effect of different concentrations of titanium dioxide nanoparticles (TiO₂ NPs) on some gram-positive bacteria, gram-negative bacteria, and *Candida albicans*. TiO₂ NPs were synthesized using the chemical methods, coated with carboxymethyl cellulose (CMC) and prepared in different concentrations (0.098, 0.196, 0.392, 0.784, 1.568, and 3.136 mg/mL). Eventually, a minimum inhibitory concentration (MIC) and a minimum biofilm inhibitory concentration (MBIC) were applied to investigate the effect of TiO₂ NPs on microorganisms.

Results: According to the study results, the MICs of TiO₂ NPs were found to be 1.489, 1.208, and 1.166 mg/mL for *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* as the Gram-negative bacteria, respectively; and they were discovered to be 0.512, 0.830, and 0.707 mg/mL for *Streptococcus pneumoniae*, *Staphylococcus aureus*, and *Staphylococcus epidermidis* as the Gram-positive bacteria, respectively. As for *C. albicans*, as the yeast strain, MIC was 0.253 mg/mL. The MBIC of more than 90% of TiO₂ NPs was 6.25 mg/mL for both Gram-negative and Gram-positive bacterial types and 1.562 mg/mL for *C. albicans*.

Conclusions: It was concluded that TiO₂ NPs were effective antimicrobial agents for Gram-positive bacteria, Gram-negative bacteria, and *C. albicans*, but their inhibitory effect on yeast was greater than that of bacteria.

Keywords: Titanium dioxide nanoparticles, Gram-positive bacteria, Gram-negative bacteria, Yeast, Biofilm

Received: 18 Feb. 2020
Accepted: 24 Nov. 2021
ePublished: 29 Dec. 2021



Background

The urinary system is one of the major vital systems in the human body, which is responsible for purifying the blood from toxic materials and returning clean blood to the circulatory system. This system, referred to as the renal system, includes the bladder, ureters, kidneys, and urethra. Microorganisms cause many infectious diseases for the human body, especially for the urinary tract system; therefore, it is essential to eliminate and control them. The emergence of antibiotic-resistant microorganisms has been increased recently, and this increase necessitates finding alternative agents to eliminate these microbes (1). Today, biofilms have become a major medical problem due to their high resistance to the immune system, as well as their resistance to antimicrobial medicine and other disinfectants (2). There are different microorganisms capable of forming a biofilm on different surfaces which can accumulate on medical devices and cause specific problems in treatment by colonization and infection (3). Most of the gram-positive bacteria, gram-negative bacteria, and yeast in nature make up the biofilm. Biofilms can cause many hospitalized infections, indicating their important role in developing infectious diseases (4). Several strains of

microbial pathogens can grow in biofilm structures. These structures can be developed by one species of microbe or a mixture of several microorganisms. Biofilms are the source of many resistant systemic infections such as urinary tract, ear, tooth, catheter, and other infections (5). Therefore, identifying factors that can inhibit the formation of biofilms has recently received extensive attention from scientists (6), including nanoparticles (NPs). The microorganisms have a high potential for adapting to new environments and antibiotics. The development in nanotechnology has provided a competitive condition against the adaptability characteristics of microorganisms and limited their growth in new media and, consequently, prevented their infections (7). The application of some nano-sized materials has shown that they have a significant effect on microbe cells (8). Many nanomaterials, such as zinc, silver, and Copper have been used to eliminate microorganisms. Haghi et al used titanium dioxide (TiO₂) NPs in dental manufacturing and showed that this NP had highly effective properties against yeast and bacteria causing teeth calcification (9). TiO₂ has received special attention in these studies for its high photocatalytic properties (10). Some of the NPs' features that make them superior to other NPs include

their high chemical resistance, non-toxicity, long service life, availability, and low cost (11). TiO₂ is widely used in many industries including water purification as well as paints, plastics, paper, and inks manufacturing industries (12). TiO₂ NPs have been applied extensively for killing different groups of microorganisms including bacteria, fungi, and viruses (13). This study, therefore, aimed to synthesize TiO₂ NPs and measure the antibacterial and antifungal effect of different concentrations of them on gram-positive bacteria, gram-negative bacteria, and *C. albicans* isolated from infected urine samples.

Materials and Methods

Bacterial Strains

To conduct the present experimental study, three strains of Gram-positive bacteria including *Streptococcus pneumoniae* PTCC 1240, *Staphylococcus aureus* PTCC 1431, and *Staphylococcus epidermidis* PTCC 1114, three Gram-negative bacteria including *Escherichia coli* PTCC 1399, *Pseudomonas aeruginosa* PTCC 1690, and *Klebsiella pneumoniae* PTCC 1290, as well as *Candida albicans* ATCC 10231 were all purchased from the Iranian Research Organization for Science and Technology.

Sample Collection

As for the sample collection, 60 urine samples (46 male and 14 female cases) were collected from the subjects with typical symptoms of a urinary tract infection. The subjects whose ages ranged from 1.5 to 72 years had been referred to Mohamad Kermanshahi Hospital in Kermanshah, Iran. It should be noted that this study lasted for six months. The men and women referring for the test were advised to clean the genital area before collecting the urine sample, discard the first part of the urine, and collect a sample of urine (mid-stream) in the container without stopping the process of urination. Patients' complete information was written on the test tubes. The samples were stored in special containers and their lids were closed and transferred to the university laboratory as soon as possible. Midstream urine samples were quickly cultured on the agar media.

The Identification of Microorganisms

For detecting microorganisms in urine samples, a loop full of samples was shed into EMB, blood agar, and potato dextrose agar and incubated at 37°C overnight. After 24 hours, individual colonies were separated and identified based on the gram-staining, morphological characteristics, and biochemical characters. To detect the gram-negative bacteria, standard biochemical tests were used, including methyl red, citrate, indole, Voges-Proskauer, H₂S production, oxidase, urea hydrolysis, and gas production, and to detect the Gram-positive bacteria, catalase, coagulase, hemolysis, motility, oxidase, DNase, and mannitol tests. Yeast isolates were identified by the germ tube test and cultured on CHROM agar media. Three strains of gram-positive bacteria including *S. pneumoniae*, *S. aureus*, and *S. epidermidis*, as well as three gram-negative

bacteria including *E. coli*, *P. aeruginosa*, *K. pneumoniae*, and *Candida albicans* were isolated (Tables 1, 2, and 3) in this experimental study (14, 15). A microbial solution was obtained from each strain according to the 0.5 McFarland standard (1×10⁸ colony forming unit/milliliter for the bacterial strain and 3×10⁶ CFU/mL for yeast) to perform the antimicrobial assays (16).

TiO₂ NP Preparation and Coating

In order to carry out this experiment, 5 mL of titanium isopropoxide was added to 8 mL deionized water, and then the precipitation of hydrous TiO₂ and alkoxide was hydrolyzed as well. The resulting solution was kept completely and mixed by persistent stirring at 40°C for half an hour. Finally, the white sediment of TiO₂ NPs was created at the bottom of the tube and then it was separated by centrifuge, washed multiple times with methanol and deionized water, and dried at 80°C in an oven for 12 hours (17,18). To prepare the photocatalytic NPs, the synthesized TiO₂ NPs were first poured into a sterilized

Table 1. Differential Tests for Gram-negative Bacteria

Biochemical Tests	Gram-negative Bacteria		
	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>K. pneumoniae</i>
Gram-staining	Negative	Negative	Negative
EMB culture	The green metallic sheen	Pink to purple in color	The absence of color
TSI	Acid/Acid	K/ -	Acid/Acid
Indole	Positive	Negative	Negative
Motility	Positive	Positive	Negative
Citrate	Negative	Positive	Positive
Urea	Negative	Negative	Positive
Lysine	Negative	No reaction	Positive
MR	Positive	Negative	Negative
Phenyl alanine	Negative	Negative	Negative

Note. EMB: Eosin methylene blue; TSI: Triple sugar iron; MR: Methyl red.

Table 2. Differential Tests for Gram-positive Bacteria

Biochemical Tests	Gram-positive Bacteria		
	<i>S. aureus</i>	<i>S. epidermidis</i>	<i>S. pneumoniae</i>
Gram-staining	Positive	Positive	Positive
Catalase	Positive	Positive	Negative
Coagulase	Positive	Negative	Negative
Mannitol	Positive	Negative	Negative
Oxidase	Negative	Negative	Negative
DNase	Positive	Negative	Positive
Hemolysis	Positive	Negative	α
Motility	Negative	Negative	Negative

Table 3. Differential Tests for *Candida albicans*

Biochemical Tests	<i>Candida albicans</i>
CHROM agar	Light to medium green colonies
Germ tube	Positive

dish and an ultraviolet lamp with a wavelength of 350 nm was placed inside a flask. Then, the output of ultraviolet rays was measured using a digital device. The measured value was $56 \mu\text{W}/\text{m}^2$. The UV lamp was then immersed in the NP solution for 60 minutes. Carboxymethyl cellulose (CMC) was used in this research to coat and improve the presentation of the TiO_2 NPs to the target microorganisms as well as to protect the nano agent from degradation (19). TiO_2 NPs were dispersed in 2% CMC and sonicated for 3 hours. For preparing 2% of CMC, 2 g of CMC was mixed with deionized water through mechanical stirring for 12 hours until a perfect dissolution was performed. The morphological and structural characteristics of these TiO_2 were analyzed using X-ray diffraction, X-ray microanalysis, transmission, and scanning electron microscopes. TiO_2 NPs used in this study were prepared at a concentration of 25 mg/mL. NPs were diluted (in DMSO-5%) in different concentrations to detect the lethal dose (LD-50). The LD-50 of TiO_2 NP responsible for the 50% death of the tested mice ($n=4$ per group) was evaluated by intraperitoneal injection with three concentrations of 0.15 $\mu\text{g}/\text{kg}$ (5 mg/mL), 0.3 $\mu\text{g}/\text{kg}$ (10 mg/mL), and 0.45 $\mu\text{g}/\text{kg}$ (15 mg/mL). Mice were monitored for body temperature, weight, mortality rate, diarrhea, drowsiness, and seizures for 2 weeks. The results of this experiment demonstrated that TiO_2 NPs at these concentrations stimulated no lethal activity after 2 weeks.

Disk Diffusion Method

For determining the antimicrobial effect of nanoparticles, the disk diffusion method was used. Briefly, the Muller-Hinton agar (MHA) medium was prepared and then the plates were inoculated with the sterile cotton swab of microorganisms. The stocks of various concentrations of TiO_2 NP were prepared using dimethyl sulfoxide 2% (DMSO, there was no antimicrobial effect in this dilution), and then 6 mm diameter sterile paper disk, *Whatman* sterilized filter paper, was used to make the disks, which were impregnated with different concentration of NPs, dried under the laminar hood and sterile condition, and placed on MHA plates. The plates were incubated at 37°C for 24 hours. The diameter of the inhibition zone, measured as a clear area around the disk, was calculated by a ruler (19). Antibiotic standards adopted in this study were purchased from Padtan Teb Company.

Minimum Inhibitory Concentrations Determination

The antimicrobial effects of TiO_2 NPs were investigated by using the micro-dilution method. In this method, 100 μm of Muller-Hinton broth medium was added to all wells. The NPs were prepared in seven concentrations (0.098, 0.196, 0.392, 0.784, 1.568, and 3.136 mg/mL). The first well in each row was filled with 100 μL of the highest concentration of TiO_2 NPs, and 100 μL of this NP was transferred to the second well, and this process was continued to the 8th well. The 8th well was adopted to control the growth of microorganisms. Finally, microplates were inoculated for

24 hours at 37°C . After that optical density (OD) of these plates was read at 600 nm and inhibitory concentration was reported in the presence of NPs (20).

Minimum Biofilm Inhibitory Concentration (MBIC) Determination

For the determination of anti-biofilm effects of nanoparticles, trypticase soy broth (TSB) medium was supplemented with 1% glucose. Different concentrations of TiO_2 NPs were added into the well of 96-well microplates (0.784, 1.568, 3.136, 6.272, and 12.544 mg/mL). The 24-hours microbial suspension was inoculated in the wells. The microplate was inoculated at 37°C for 24 hours (21). The cultures were then aspirated and the wells were washed three times with phosphate-buffered saline. Next, the plates were air-dried overnight and stained with 0.1% crystal violet. The OD of the wells was measured at 600 nm using the ELISA reader device.

Results

Disk Diffusion Method

MHA was used for antimicrobial susceptibility testing of the mentioned TiO_2 NPs. Anti-bacterial disks of the CMC-coated TiO_2 NP were evaluated for inhibition of microbial growth of the UTI-causing bacterial agents. In this step, concentrations from 1.568, 3.136, 6.272, and 12.544 mg/mL were used. The zone of bacterial growth inhibition was inspected after planting the discs on the Muller-Hinton media. Evaluating the zone of inhibition after 24 hours showed that the prepared nano-agent was effectively capable of suppressing the bacterial growth on all UTI-causing bacteria at all different TiO_2 concentrations. In Table 4, the diameter of the zone of inhibition on the bacterial species was represented in millimeters. The results of the disk diffusion method using standard antibiotic disks demonstrated that *E. coli* had the highest resistance to antibiotics ampicillin, while cephalothin and cotrimoxazole had the highest sensitivity to antibiotics gentamicin and amikacin. *P. aeruginosa* exhibited the highest resistance to antibiotics ciprofloxacin and the highest sensitivity to gentamicin. *Klebsiella* represented the highest resistance to antibiotics ampicillin and the highest sensitivity to antibiotics gentamicin and amikacin. *S. pneumonia* had the highest resistance to antibiotics penicillin and the highest sensitivity to antibiotics erythromycin. *S. aureus* developed the highest resistance to antibiotics ceftriaxone and cefixime, as well as the highest sensitivity to antibiotics cotrimoxazole. *S. epidermis* had the highest resistance to antibiotics oxacillin and the highest sensitivity to antibiotics cephalothin.

Characteristics of TiO_2 NPs

Details of this component are TiO_2 , anatase, average particle size of 10-25 nm, the specific surface area of 200-240 m^2/g , pH rate of 6-6.5, white color, purity of $>99\%$, bulk density of 0.24 g/cm^3 , true density of 3.9 g/cm^3 , loss of weight in drying of 4.17%, and loss of weight on ignition

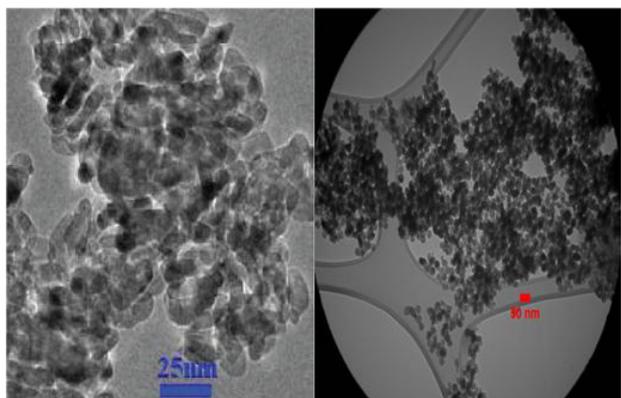


Figure 1. SEM (left) and TEM (right) Imaging of TiO₂ NPs. Note. SEM: Scanning electron microscopy; TEM: Transmission electron microscopy; TiO₂ NPs: Titanium dioxide nanoparticles. The TEM image shows that loaded NPs could efficiently internalize into the target cells.

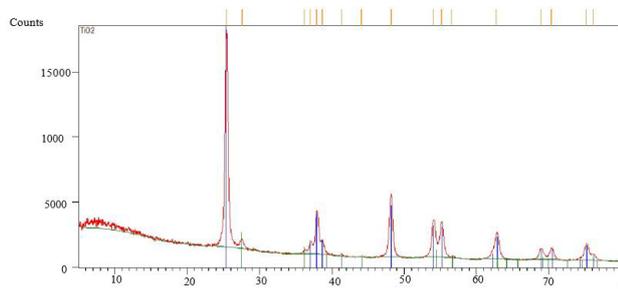


Figure 2. EDX Characteristics of the TiO₂ Nanoparticles. Note. EDX: X-ray microanalysis; TiO₂: Titanium dioxide nanoparticles.

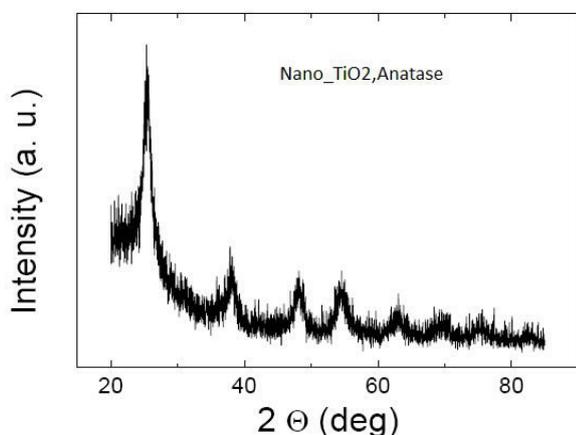


Figure 3. XRD Characteristics of TiO₂ Nanoparticles. Note. XRD: X-ray diffraction; TiO₂: Titanium dioxide nanoparticles.

equal to 8.24% (Figures 1-3).

Minimum Inhibitory Concentration Results

In this test, the MIC of nanoparticles was determined by the microdilution method for *S. pneumoniae*, *S. aureus*, *S. epidermidis*, *E. coli*, *P. aeruginosa*, *K. pneumoniae*, and *C. albicans*. Microbial growth rates in the presence of different concentrations of TiO₂ NPs were calculated in 600 nm OD (Tables 5-7). For MBC detection, two wells before and after the MIC well cultured on Mueller Hinton agar media. Table 8 presents the MIC test results for the microbial strains in the current study. As shown in the table,

these particles were capable of producing an acceptable inhibitory effect on all examined groups of bacteria and fungi. The result revealed that the MICs of TiO₂ NPs for *E. coli*, *P. aeruginosa*, and *K. pneumoniae*, as Gram-negative bacteria, were 1.489, 1.208, and 1.166 mg/mL, respectively. The corresponding values for *S. pneumoniae*, *S. aureus*, and *S. epidermidis* as gram-positive bacteria were 0.512, 0.830, and 0.707 mg/mL, respectively. Finally, this value was 0.253 mg/mL for *C. albicans*, as the yeast strain. The cultured plates of microbes representing no microbial growth were considered MBC/MFC (Table 8). Thus, TiO₂ NPs are effective antimicrobial agents for gram-positive, Gram-negative bacteria, and *C. albicans*, but their inhibitory effect on yeast was greater than bacteria.

Minimum Biofilm Inhibitory Concentration

The results of biofilm inhibition assay using CMC coated

Table 4. Antimicrobial effect of Different Concentrations of Titanium Dioxide Nanoparticles by the Disk Diffusion Method

Microorganisms	Concentration (mg/mL)		
	3.136	6.272	12.544
<i>E. coli</i>	10±0.7	10.5±0.8	11.7±0.4
<i>P. aeruginosa</i>	9.6±0.7	9.6±0.7	10.3±0.6
<i>K. pneumoniae</i>	9.5±0.4	10.1±0.7	11.2±0.2
<i>S. aureus</i>	10.1±0.1	10.8±0.9	12.2±0.6
<i>S. epidermidis</i>	10.4±0.9	10.9±0.5	12.1±0.4
<i>S. pneumoniae</i>	0	9.6±0.7	10.3±0.6
<i>C. albicans</i>	11.1±1.2	11.4±0.9	12.5±1.2

Table 5. Gram-Negative Bacteria Growth Rate in the Presence of Different Concentrations of Titanium Dioxide Nanoparticles in 600 nm Optical Density

Concentration (mg/mL)	Gram-Negative Bacteria		
	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>K. pneumoniae</i>
0 (Control)	0.727±0.005	0.601±0.001	0.855±0.003
0.098	0.572±0.014	0.579±0.003	0.712±0.005
0.196	0.485±0.004	0.438±0.003	0.643±0.004
0.392	0.356±0.003	0.219±0.011	0.442±0.004
0.784	0.233±0.004	0.157±0.001	0.342±0.002
1.568	0.098±0.003	-0.013±0.002	0.103±0.002
3.136	-0.033±0.001	-0.029±0.006	-0.005±0.003

Table 6. Gram-positive Bacteria Growth Rate in the Presence of Different Concentrations of Titanium Dioxide Nanoparticles in 600 nm Optical Density

Concentration (mg/mL)	Gram-Positive Bacteria		
	<i>S. aureus</i>	<i>S. epidermidis</i>	<i>S. pneumoniae</i>
0 (Control)	0.718±0.002	0.851±0.004	0.632±0.004
0.098	0.706±0.003	0.535±0.003	0.544±0.003
0.196	0.691±0.001	0.465±0.007	0.508±0.005
0.392	0.654±0.005	0.236±0.005	0.363±0.005
0.784	0.384±0.005	0.204±0.003	0.227±0.003
1.568	-0.029±0.002	0.012±0.003	0.096±0.004
3.136	-0.037±0.001	-0.015±0.003	0.039±0.004

Table 7. Yeast Growth Rate in the Presence of Different Concentrations of Titanium Dioxide Nanoparticles in 600 nm Optical Density

Concentration (mg/mL)	<i>Candida albicans</i>
0 (Control)	0.571±0.008
0.098	0.552±0.002
0.196	0.365±0.004
0.392	0.044±0.005
0.784	-0.011±0.003
1.568	-0.036±0.003
3.136	-0.039±0.002

Table 8. MIC of Titanium Dioxide Nanoparticles Against Yeast, Gram-Negative, and Gram-Positive Bacteria

Microorganisms	MIC (mg/mL)	MBC/MFC (mg/mL)
<i>E. coli</i>	1.489	3.136
<i>P. aeruginosa</i>	1.208	3.136
<i>K. pneumoniae</i>	1.166	3.136
<i>S. aureus</i>	0.830	1.568
<i>S. epidermidis</i>	0.707	1.568
<i>S. pneumoniae</i>	0.512	1.568
<i>C. albicans</i>	0.253	0.784

Note. MIC: Minimum inhibitory concentration; MBC: Minimum bactericidal concentration; MFC: Minimum fungicidal concentration.

TiO₂ NPs on 96-well plates showed that these NPs are completely capable of suppressing biofilm formation, and preventing further growth of the microbial biofilm of *S. pneumoniae*, *S. aureus*, *S. epidermidis*, *E. coli*, *P. aeruginosa*, *K. pneumoniae*, and *C. albicans*. The test results indicated that in the absence of the TiO₂ NPs, strong biofilms will form on the plates, but adding the particles would strongly inhibit biofilm formation and maturation. TiO₂ demonstrated that it could effectively inhibit the growth of all isolated bacterial strains at concentrations of 25-0.39 mg/mL. Based on the result in Tables 9-11, the MBIC of more than 90% of TiO₂ NPs was 6.25 mg/mL for both Gram-negative and Gram-positive bacterial types and 1.562 mg/mL for *C. albicans*.

Discussion

In recent years, physicians and microbial scientists have found that the causative agents of microbial infections are becoming resistant to common antimicrobial medicine. On the other side, by the acceleration of science and a better understanding of new materials with the potential for treating infection, many have focused on studying the antimicrobial properties of these materials in order to find new substitutions for ordinary antibiotics (22). As previously stated, biofilms are a major cause of infectious diseases and could increase the tolerance of bacteria inside them toward different antibiotics (23). Therefore, finding a drug that could penetrate into the biofilm and destroy them, would be helpful for effectively battling infections. Inhibition of biofilm-associated diseases requires incredible attention since this structure has high levels of

resistance to antibiotics (24). Several studies recommended combination therapy as the treatment of choice in biofilm-associated infections while considering macrolides as one of the first selected antibiotics, but many of these microbial agents of biofilm construction have become resistant to them over time. Biofilms can be up to multiple-fold more resistant to antimicrobial agents compared to planktonic forms due to various mechanisms (25). The current time could be considered as the age of nanotechnology for its fascinating applications and encouraging results of their use for improving different aspects of human life. An understanding of NPs affecting the critical causative agent of microbial infections allows us to gain a mechanistic understanding of toxicity and guides us toward designing rules for creating safe antimicrobial nanomaterials (26). As mentioned earlier, an NP is a small-sized particle with a dimension of less than 100 nm. NP studies are a field of intensive research interest due to a broad diversity of potential applications in electronic, biomedical, and optical fields. These particles have the capacity to attach and penetrate into bacterial cells, disrupt the bacterial membrane, and interact with chromosomal DNA (27).

Table 9. Percentage of Gram-Negative Biofilm Inhibition in Different Concentrations of Titanium Dioxide Nanoparticles in 600 nm Optical Density

Concentration (mg/mL)	Gram-Negative Bacteria		
	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>K. pneumoniae</i>
1.568	47.3	21.62	48.56
0.784	51.08	24.60	65.29
1.568	69.29	71.02	74.77
3.136	80.82	80.07	89.89
6.272	92.41	91.12	93.29
12.544	100	100	100

Table 10. Percentage of Gram-Positive Biofilm Inhibition in Different Concentrations of Titanium Dioxide Nanoparticles in 600 nm Optical Density

Concentration (mg/mL)	Gram-positive Bacteria		
	<i>S. aureus</i>	<i>S. epidermidis</i>	<i>S. pneumoniae</i>
1.568	23.01	48.81	53.10
0.784	45.71	63.26	73.11
1.568	74.81	70.13	77
3.136	81.12	80.79	83.87
6.272	92.82	100	100
12.544	99.76	100	100

Table 11. Percentage of Yeast Biofilm Inhibition in Different Concentrations of Titanium Dioxide Nanoparticles in 600 nm Optical Density

Concentration (mg/mL)	<i>Candida albicans</i>
1.568	57.24
0.784	84.02
1.568	90.38
3.136	100
6.272	100
12.544	100

The purpose of this research was to provide new insights about using CMC-coated photocatalytic TiO₂ NPs as antimicrobial agents against different gram-positive, gram-negative bacteria, and *C. albicans*.

In this study, the antimicrobial effect of CMC-coated photocatalytic TiO₂ NPs was investigated on seven common agents of many infections, including *S. pneumoniae*, *S. aureus*, *S. epidermidis*, *E. coli*, *P. aeruginosa*, *K. pneumoniae*, and *C. albicans*. The antibacterial effects of these materials were evaluated using the microdilution method. The biofilm inhibition effects of TiO₂-coated NPs were determined as well. The results of our test indicated that strong biofilms will form on the plates in the absence of the TiO₂ NPs, but adding the particles would strongly inhibit biofilm formation and maturation. The result showed that the MICs of TiO₂ NPs for *E. coli*, *P. aeruginosa*, and *K. pneumoniae*, as the Gram-negative bacteria, were 1.489, 1.208, and 1.166 mg/mL, respectively. Further, these values were 0.512, 0.830, and 0.707 mg/mL for *S. pneumoniae*, *S. aureus*, and *S. epidermidis* as the Gram-positive bacteria, respectively. Moreover, it was equal to 0.253 mg/mL for *C. albicans* as the yeast strain. The MBIC of more than 90% of TiO₂ NPs was 6.25 mg/mL for both Gram-negative and Gram-positive bacterial types and 1.562 mg/mL for *C. albicans*. In their study, Jalvo et al focused on measuring the antibiofilm and antimicrobial properties of functionalized surfaces coated by photocatalytic TiO₂ NPs against *Pseudomonas putida* and *S. aureus*. They presented that the antimicrobial activity of TiO₂ led to wide cover damage. The decrease of cell inviability was more than 99.9% for TiO₂ NPs on the glass level (21). In another study, Verdier et al examined the antimicrobial effect of photocatalytic TiO₂ alone or in coatings on *E. coli* bacterium and measured various types of parameters that significantly influence the antimicrobial activity (28). Similarly, Lavaee et al reported the results of investigating the antibacterial and biofilm inhibition activity of silver, TiO₂, and iron NPs. They aimed at comparing the antibacterial effects of erythromycin, clindamycin, chlorhexidine, penicillin, vancomycin, and tetracycline with TiO₂, iron, and Ag NPs and consider the synergistic antimicrobial and biofilm inhibition effects of these NPs in pathogenic and standard species of *Streptococcus sanguinis* and *Streptococcus mutans*. Based on their findings, the use of NPs alone showed a higher MIC than using them synergistically. The most efficient synergistic resolving agent was the one containing Ag, TiO₂, and Fe₃O₄ (29). In another study, Vincent et al evaluated the efficacy of TiO₂ and zinc oxide nanomaterials against biofilm-producing and metallo beta-lactamase *P. aeruginosa* aiming at determining the effect of TiO₂ and zinc oxide NPs on biofilm-producing and metallo beta-lactamase *P. aeruginosa*. These nanomaterials demonstrated perceptible activity at whole experimental concentrations, and it was revealed that TiO₂ and zinc oxide nanomaterials may serve as promising antimicrobial agents in this regard (30).

Conclusions

Overall, the results of the current study demonstrated that TiO₂ NPs are effective antimicrobial agents for *E. coli*, *P. aeruginosa*, and *K. pneumoniae*, as Gram-negative bacteria, as well as *S. pneumoniae*, *S. aureus*, and *S. epidermidis*, as Gram-positive bacteria, and *C. albicans*, as the yeast strain, and repressed biofilm composition for the first time. In addition, the findings confirmed that the efficiency for the inhibition of both microbial and fungi biofilms is of great importance for these particles and they could be used against a diversity of infections. Although the results of the present study seem to be successful, further studies are required so that to consider these agents for clinical applications.

Acknowledgements

This paper is extracted from the MSc thesis of the first author submitted to the Department of Biology, Faculty of Science, Razi University (67149-67346), Kermanshah, Iran. The authors acknowledge the personnel of the laboratory of microbiology for technical assistance.

Conflict of Interests

None declared.

Ethical Approval

In this experimental study, Ethical principles were considered in relation to the proposed work.

Financial Support

This study was supported by intramural funds.

References

- Jang HD, Kim SK, Kim SJ. Effect of particle size and phase composition of titanium dioxide nanoparticles on the photocatalytic properties. *J Nanopart Res.* 2001;3(2):141-7. doi: [10.1023/A:1017948330363](https://doi.org/10.1023/A:1017948330363).
- Talpaert MJ, Balfour A, Stevens S, Baker M, Muhlschlegel FA, Gourlay CW. *Candida* biofilm formation on voice prostheses. *J Med Microbiol.* 2015;64(Pt 3):199-208. doi: [10.1099/jmm.0.078717-0](https://doi.org/10.1099/jmm.0.078717-0).
- Flemming HC, Wingender J, Szewzyk U, Steinberg P, Rice SA, Kjelleberg S. Biofilms: an emergent form of bacterial life. *Nat Rev Microbiol.* 2016;14(9):563-75. doi: [10.1038/nrmicro.2016.94](https://doi.org/10.1038/nrmicro.2016.94).
- Butterfield PW, Bargmeyer AM, Camper AK, Biederman JA. Modified enzyme activity assay to determine biofilm biomass. *J Microbiol Methods.* 2002;50(1):23-31. doi: [10.1016/s0167-7012\(02\)00005-2](https://doi.org/10.1016/s0167-7012(02)00005-2).
- Kojic EM, Darouiche RO. *Candida* infections of medical devices. *Clin Microbiol Rev.* 2004;17(2):255-67. doi: [10.1128/cmr.17.2.255-267.2004](https://doi.org/10.1128/cmr.17.2.255-267.2004).
- Habimana O, Steenkeste K, Fontaine-Aupart MP, Bellon-Fontaine MN, Kulakauskas S, Briandet R. Diffusion of nanoparticles in biofilms is altered by bacterial cell wall hydrophobicity. *Appl Environ Microbiol.* 2011;77(1):367-8. doi: [10.1128/aem.02163-10](https://doi.org/10.1128/aem.02163-10).
- Nana A, Nelson SB, McLaren A, Chen AF. What's new in musculoskeletal infection: update on biofilms. *J Bone Joint Surg Am.* 2016;98(14):1226-34. doi: [10.2106/jbjs.16.00300](https://doi.org/10.2106/jbjs.16.00300).
- Lin X, Li J, Ma S, Liu G, Yang K, Tong M, et al. Toxicity of TiO₂ nanoparticles to *Escherichia coli*: effects of particle size, crystal phase and water chemistry. *PLoS One.* 2014;9(10):e110247. doi: [10.1371/journal.pone.0110247](https://doi.org/10.1371/journal.pone.0110247).
- Haghi F, Haghi M, Maadi H, Charkhiyan H, Ghahramani A.

- The antibacterial effect of TiO₂ nanoparticles on pathogenic strain of *Escherichia coli*, in vitro. In: The First International Congress of Medical Bacteriology. Tabriz University of Medical Sciences; 2011.
10. Jeena V, Robinson RS. Green oxidations: titanium dioxide induced tandem oxidation coupling reactions. *Beilstein J Org Chem*. 2009;5:24. doi: [10.3762/bjoc.5.24](https://doi.org/10.3762/bjoc.5.24).
 11. Enyashin AN, Seifert G. Structure, stability and electronic properties of TiO₂ nanostructures. *Phys Status Solidi B*. 2005;242(7):1361-70. doi: [10.1002/pssb.200540026](https://doi.org/10.1002/pssb.200540026).
 12. Wu X, Liu H, Liu J, Haley KN, Treadway JA, Larson JP, et al. Immunofluorescent labeling of cancer marker Her2 and other cellular targets with semiconductor quantum dots. *Nat Biotechnol*. 2003;21(1):41-6. doi: [10.1038/nbt764](https://doi.org/10.1038/nbt764).
 13. Gupta K, Singh RP, Pandey A, Pandey A. Photocatalytic antibacterial performance of TiO₂ and Ag-doped TiO₂ against *S. aureus*, *P. aeruginosa* and *E. coli*. *Beilstein J Nanotechnol*. 2013;4:345-51. doi: [10.3762/bjnano.4.40](https://doi.org/10.3762/bjnano.4.40).
 14. Costa AR, Silva F, Henriques M, Azeredo J, Oliveira R, Faustino A. *Candida* clinical species identification: molecular and biochemical methods. *Ann Microbiol*. 2010;60(1):105-12. doi: [10.1007/s13213-009-0007-6](https://doi.org/10.1007/s13213-009-0007-6).
 15. Franco-Duarte R, Černáková L, Kadam S, Kaushik KS, Salehi B, Bevilacqua A, et al. Advances in chemical and biological methods to identify microorganisms-from past to present. *Microorganisms*. 2019;7(5):130. doi: [10.3390/microorganisms7050130](https://doi.org/10.3390/microorganisms7050130).
 16. Weinstein MP, Lewis JS 2nd. The clinical and laboratory standards institute subcommittee on antimicrobial susceptibility testing: background, organization, functions, and processes. *J Clin Microbiol*. 2020;58(3):e01864-19. doi: [10.1128/jcm.01864-19](https://doi.org/10.1128/jcm.01864-19).
 17. Buraso W, Lachom V, Siriya P, Laokul P. Synthesis of TiO₂ nanoparticles via a simple precipitation method and photocatalytic performance. *Mater Res Express*. 2018;5(11):115003. doi: [10.1088/2053-1591/aadbf0](https://doi.org/10.1088/2053-1591/aadbf0).
 18. Lachom V, Poolcharuansin P, Laokul P. Preparation, characterizations and photocatalytic activity of a ZnO/TiO₂ nanocomposite. *Mater Res Express*. 2017;4(3):035006. doi: [10.1088/2053-1591/aa60d1](https://doi.org/10.1088/2053-1591/aa60d1).
 19. Olajuyigbe OO, Afolayan AJ. In vitro antibacterial and time-kill assessment of crude methanolic stem bark extract of *Acacia mearnsii* de wild against bacteria in shigellosis. *Molecules*. 2012;17(2):2103-18. doi: [10.3390/molecules17022103](https://doi.org/10.3390/molecules17022103).
 20. Wiegand I, Hilpert K, Hancock RE. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nat Protoc*. 2008;3(2):163-75. doi: [10.1038/nprot.2007.521](https://doi.org/10.1038/nprot.2007.521).
 21. Jalvo B, Faraldos M, Bahamonde A, Rosal R. Antimicrobial and antibiofilm efficacy of self-cleaning surfaces functionalized by TiO₂ photocatalytic nanoparticles against *Staphylococcus aureus* and *Pseudomonas putida*. *J Hazard Mater*. 2017;340:160-70. doi: [10.1016/j.jhazmat.2017.07.005](https://doi.org/10.1016/j.jhazmat.2017.07.005).
 22. Penesyan A, Gillings M, Paulsen IT. Antibiotic discovery: combatting bacterial resistance in cells and in biofilm communities. *Molecules*. 2015;20(4):5286-98. doi: [10.3390/molecules20045286](https://doi.org/10.3390/molecules20045286).
 23. Al-Badr A, Al-Shaikh G. Recurrent urinary tract infections management in women: a review. *Sultan Qaboos Univ Med J*. 2013;13(3):359-67. doi: [10.12816/0003256](https://doi.org/10.12816/0003256).
 24. Musk DJ Jr, Hergenrother PJ. Chemical countermeasures for the control of bacterial biofilms: effective compounds and promising targets. *Curr Med Chem*. 2006;13(18):2163-77. doi: [10.2174/092986706777935212](https://doi.org/10.2174/092986706777935212).
 25. Davies D. Understanding biofilm resistance to antibacterial agents. *Nat Rev Drug Discov*. 2003;2(2):114-22. doi: [10.1038/nrd1008](https://doi.org/10.1038/nrd1008).
 26. Renn O, Roco MC. Nanotechnology and the need for risk governance. *J Nanopart Res*. 2006;8(2):153-91. doi: [10.1007/s11051-006-9092-7](https://doi.org/10.1007/s11051-006-9092-7).
 27. Kamaruzzaman NF, Tan LP, Mat Yazid KA, Saeed SI, Hamdan RH, Choong SS, et al. Targeting the bacterial protective armour; challenges and novel strategies in the treatment of microbial biofilm. *Materials (Basel)*. 2018;11(9):1705. doi: [10.3390/ma11091705](https://doi.org/10.3390/ma11091705).
 28. Verdier T, Coutand M, Bertron A, Roques C. Antibacterial activity of TiO₂ photocatalyst alone or in coatings on *E. coli*: the influence of methodological aspects. *Coatings*. 2014;4(3):670-86. doi: [10.3390/coatings4030670](https://doi.org/10.3390/coatings4030670).
 29. Lavaee F, Ghapanchi J, Motamedifar M, Sorourian S. An in vitro analysis of the effects of iron sulfate and iron acetate on *Streptococcus mutans*. *J Dent Biomater*. 2018;5(1):528-32.
 30. Vincent MG, John NP, Narayanan PM, Vani C, Murugan S. In vitro study on the efficacy of zinc oxide and titanium dioxide nanoparticles against metallo beta-lactamase and biofilm producing *Pseudomonas aeruginosa*. *J Appl Pharm Sci*. 2014;4(7):41-6. doi: [10.7324/japs.2014.40707](https://doi.org/10.7324/japs.2014.40707).