Research Article

Titanium dioxide nanoparticles as antibacterial agents against some pathogenic bacteria

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ABSTRACT

Background: Today, many of studies have been done to investigate the efficacy of antimicrobial nanoparticles (NPs) against the pathogens as drug resistance bacteria and metal NPs have been reported with antimicrobial properties. **Materials and Methods:** In the present study, titanium dioxide (TiO_2) NPs with 35 nm size were characterized by X-ray diffraction and Fourier transform infrared then investigates their antibacterial activity, antibiofilm formation, and invasion against various pathogenic bacteria from Gram-positive *Streptococcus pyogenes* and *Staphylococcus aureus* and Gram-negative *Pseudomonas aeruginosa, Acinetobacter baumannii, Escherichia coli, Proteus vulgaris,* and *Serratia marcescens*. Antimicrobial activity of TiO_2 NPs was examined by disk diffusion assay using dilutions of 500, 250, 125, 62.5, and 31.25 µg/ml also the minimal inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of each isolate is determined. **Results:** TiO_2 NPs show powerful broad-spectrum antibacterial activity against tested bacteria with increase in inhibition zone diameter that is directly proportional with the increase in NP concentration that even exceeded the activity of selected antibiotics. The MIC of TiO_2 NPs ranged from 125 µg/ml to 31.25 µg/ml and the MBC ranged from 125 µg/ml to 500 µg/ml. The metal NPs highly inhibited bacterial biofilm growth and invasion, other studies show that TiO_2 NPs strongly attached to the bacterial cells that contributed to their inhibitory effect on bacterial biofilm growth formation and invasion. We showed that bacterial biofilm growth was reduced at MIC concentrations of TiO_2 NPs compared with another test without the NPs. **Conclusion:** NPs with a suitable concentration are reduced the biofilm growth significantly. It is highly recommended using TiO_2 NPs as an economic alternative antibacterial and antibiofilm agent, especially in treating ectopic infections without taking the risk of developing resistant bacterial strains as with antibiotics.

KEY WORDS: Antibacterial activity, Biofilm, Nanoparticles, Titanium dioxide

INTRODUCTION

Nanobiotechnology, a modern field of nanoscience, utilizes nanobased as systems for different biomedical field and applications. Metal nanoparticles (NPs) have a largely surface area to volume ratio and a high pieces on surface atoms have been studied widely due to the unique physicochemical characteristics including catalytic activity, electronic properties, optical properties, magnetic properties, and antibacterial properties.[1-3]

As titanium dioxide (TiO_2) NPs, these days have attracting a great deal of interest because it has properties to achieve highly effect in biological, pharmaceutical applications, purification of environmental source, electronic system, cells of solar energy, photocatalysts, photoelectrodes, and sensors of gas beside American Food and Drug Administration acceptance for using in food technology and drugs, paints pigment, cosmetics, ointments, and toothpaste.[4] TiO_2 is occurred naturally and has many properties such as high refractive index, absorption of light, free toxicity, stability, and less cost production.[5] TiO_2 has three crystalline phases: anatase, rutile, and brookite. Thermodynamically, rutile is the more stable shape. Anatase very attracted more interest due to its uses in photovoltaic cells[6] and photocatalysts[7] and for its antimicrobial properties. Nowadays, TiO_2 is widely applied as a self-cleaning and self-antiseptic surface covering materials, TiO_2 has a more application

Access this article online

Website: jprsolutions.info | ISSN: 0975-7619

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Received on: 19-12-2018; Revised on: 21-01-2019; Accepted on: 18-02-2019

in purification, the environmental due to its photo has ability to induced superhydrophobicity and antifogging activity.[8] These specifications have been utilized as antibacteria and remove different materials from air and water, especially harmful organic material, also their capacity as self-cleaning or self-antiseptic surfaces in different medical institutes.[9] On the other hand, TiO_2 can synthesize by different methods such as biological and chemical process, has been appeared to be biocompatible and appropriate supports for a broad variety of compounds.[10] The effect of TiO_2 NPs on microorganism is extreme importance due to ability of pathogenic microorganism to attack in ecosystem food chain[11] and activate free hydroxyl radicals by TiO_2 NPs.[12] The antimicrobial effect of TiO_2 against fungi and bacteria has been demonstrated[13] and communicating in modern research. The objective of recent study was used the TiO_2 NPs to study the antibacterial and antibiofilm activity on some Gram-negative (G−ve) and Gram-positive (G+ve) bacteria to anticipate information to improve new antimicrobial disinfecting solutions can be used as effective on bacterial biofilm in science technology.

MATERIALS AND METHODS

Bacterial Isolates

The bacterial isolates were obtained from the patients in Teaching Hospital in Hillah, Iraq. All samples were subjected to standard bacteriological processes of culturing on blood and MacConkey's agar plates for 24–48 h at 37°C for isolation and purification. All isolates were confirmed by Vitek 2 compact system (Biomérieux).

Solution and Media

Mueller-Hinton agar and Mueller-Hinton media were obtained from Hi-Media, Mumbai, India. Titanium oxide TiO_2 NPs (35 nm) were supplied from (Zhengzhou Dongyao Nano Materials Co., Ltd., China). Different antibiotic disks ciprofloxacin (CIP) (CIP-10), erythromycin (E-15), gentamicin (CN10), meropenem (ME-5), penicillin (P-10), and tetracycline (TE-30) were purchased from (Bioanalyse, Turkey). TiO_2 was supplied from (Sigma-Aldrich).

Characterization of TiO_2 NPs

The TiO_2 NP was confirmed by characterized these NPs by Fourier transform infrared (FTIR) measurements and reflectance X-ray diffraction measurements (XRD).

Antibacterial Activity of TiO_2 NPs

TiO_2 NPs antimicrobial activity was tested against some human pathogenic bacteria, five G−ve bacteria (*Pseudomonas aeruginosa, Serratia marcescens, Escherichia coli, Acinetobacter baumannii,* and *Proteus vulgaris*) with two G+ve bacteria (*Streptococcus pyogenes* and *Staphylococcus aureus*) that were maintained on nutrient agar slants. The antimicrobial activity was carried out as described by the Clinical and Laboratory Standards Institute.[14] Antibiotic sensitivity and TiO_2 NPs against bacteria under study are tested using a disk diffusion assay, with triplicates used in dilutions of concentration of TiO_2 NPs (500, 250, 125, 62.5, and 31.25 µg/ml) in sterile deionized water. The first step, the isolates were incubated for 15 min at room temperature, then incubated at 37°C overnight. Positive results were recorded when the inhibition zone was observed around the well after a period of incubation, and then, the inhibition zone diameter was measured using a digital Vernier caliper.[15]

Determination the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The bacterial isolates were incubated at 37°C overnight, which were used to prepare 0.5 McFarland. The total of 10 ml tube nutrient broth medium was prepared, and then, each sample was inoculated aseptically with 1 ml of the bacterial suspension (about 108 colony-forming unit/mL). Five dilutions of TiO_2 NPs were prepared (500, 250, 125, 62.5, and 31.25 µg/ml) in sterile deionized water and a negative control (without TiO_2 NPs) was used. Each isolate was tested performed in triplicates using multiplate count. The inoculated plates were incubated at 37°C overnight. The MIC was determined using the spectrophotometry at wavelength 600 nm after incubation period.[16] Wells showed that no turbidity was cultured on nutrient agar plates and incubated at 37°C overnight. Bacterial colonies growth was checked and the concentration that shows no growth is represented as the MBC.

Effect of NPs on Bacteria Biofilms

Bacteria isolate at the beginning and incubating at 37°C overnight on blood agar plates by taken inoculum from the stock putted in the freeze. The prepared plate was stored at 4°C. For each experiment, only one colony from the plate was inoculated in 10 mL of tryptone soy broth (TSB) then cultured in the incubation for 16 h. The bacteria are suspended in tube of TSB to prepare a concentration of 10.6 bacteria/mL.

Multiwells microtiter plate (12 wells) were filled by 1 mL of bacterial suspension under study and let go to grow and better grow under aerobic condition 37°C for 4 h. Then, TiO_2 NPs were prepared in different concentrations (500, 250, 125, 62.5, and 31.25 µg/mL), then allowed the biofilms of bacteria to grow for 24 h. Thereafter, washing all wells with sterile distilled water, to remove all unbounded bacteria, dried under room temperature, then stained using 125 µl of crystal violet dye solution 0.1% for about

10–15 min. After the staining, all wells were washed as 3 times using sterile distilled water and then removing the stained using 125 µl of 30% acetic acid dissolved in water. A new sterile, multiwell microtiter plate with flat-bottomed was prepared with 125 µl destaining solution in each well. Measuring the absorbance of the destaining solution was measured at 630 nm using the ELISA reader (Stat Fax-2100). Each test was performed in triplicate,and thecontrol (onlymedium) was used. Classification of bacterial biofilm formation by tissue culture plate method in to three categories: Weak (BF <0.120),Moderat(0.120> BF ≤0.240), and Strong (BF >0.240) at OD. value 630 nm.[17] Data are documented as removing completely and incompletely in the biofilm bacterial growth with presence of TiO_2 nanoparticles and compared with the absence of TiO_2 nanoparticles (control).

RESULTS AND DISCUSSION

FTIR spectra of TiO_2 NPs under test are as shown in Figure 1. Peaks at 3429.55 cm^{-1} and 3225.09 cm^{-1} matching to stretching vibration of O-H bond. Peaks showed at 2048.47 cm^{-1} matching to the C-H stretching vibrations. The peak at 1649.19 cm^{-1} corresponds to the C = O vibrations. Observation of peaks at 651.96 cm^{-1}, 584.45 cm^{-1}, and 569.02 cm^{-1} corresponds to Ti-O vibrations.[18]

Figure 2 shows the XRD patterns of the TiO_2 NPs sample, peaks at 25°, 37°, 47°, 53°, 54°, 63°, and 68° (JCPDS-781510) the observation which confirms to the formation of anatase stage of TiO_2 NPs and corresponds to various crystalline planes. The sharp diffraction patterns showed to the small size of NPs, crystallinity shape, and high purity of the TiO_2 NPs synthesized sample.[19]

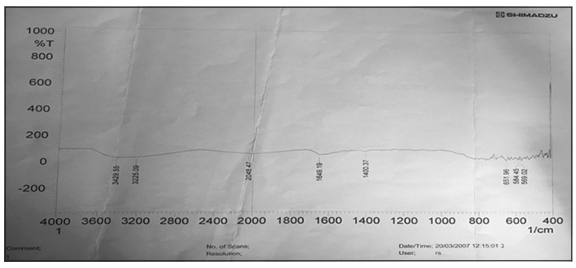

Figure 1: Fourier transform infrared spectra of TiO_2 nanoparticles

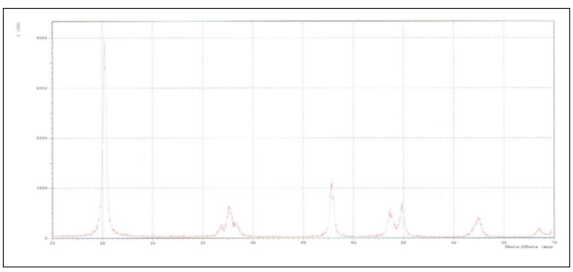

Figure 2: X-ray diffraction of TiO_2 nanoparticles

Antibacterial Activity of TiO_2 NPs

TiO_2 NPs shows that powerful broad-spectrum antibacterial activity against multidrug bacteria is tested. The effects of different antibiotics on bacterial isolates were compared. The result in Table 1 showed that the selected antibiotics were not effective against all elected bacterial strains. TiO_2 NPs showed clearly inhibition zone diameter with the increase in NP concentration that even exceeded the activity of selected antibiotics. 500 µg/ml concentration showed highest zone of inhibition against the test organisms, maximum zone of inhibition of 24 mm appeared against *Streptococcus pyogenes* that showed high sensitivity even at 31.25 µg/ml. *P. vulgaris* the least sensitive isolate in comparison with the selected antibiotics followed by *E. coli* [Table 2]. TiO_2 NPs cause sudden decline in bacterial cell membrane integrity in addition to the release of reactive oxygen species where superoxide species is generated and contributing in the degradation of biomolecules.[20]

Minimal residual disease was defined as acquired non-susceptibility to at least one agent among three or more antibacterial antibiotics or categories.[14]

Table 2 shows that significant increase ($P \leq 0.05$) in all TiO_2 NPs was used against bacterial isolates are used under study. The result has been agreed with the Zhang and Chen[11] shown that TiO_2 NPs could be inhibited the multidrug-resistant (MDR) bacteria.

MIC and MBC [Table 3] show that the MIC of TiO_2 NPs ranged from 31.25 µg/ml to 125 µg/ml and the MBC ranged from 125 µg/ml to 500 µg/ml where showed *A. baumannii* and *S. pyogenes* highest sensitivity.[21]

Antibiofilm Activity of TiO_2 NPs

In vitro experimental data showed the effect of TiO_2 NPs significantly on bacterial biofilm growth. The differences may observe due to the form of NPs, type of bacteria cell wall, and NP concentrations. At a concentration of 500 µg/mL, TiO_2 NPs showed reduction biofilm growth completely in *P. vulgaris* and *S. aureus* (MBC con.), whereas a concentration of 125 µg/mL TiO_2 NPs showed reduction biofilm growth completely in *A. baumannii* and *S. pyogenes* (MBC con.) [Table 4].

The biofilm bacterial growth inhibited by TiO_2 NPs is related to specific bacteria isolates or due to growth conditions, G−ve bacteria and G+ve bacteria varied in their capacity to produce their biofilm on the different surface of material. In most studies , the amount of dye (crystal violet)removed from G-ve biofilmswas

Table 1: Antibiotic sensitivity for some pathogenic bacterial isolate

Bacterial strain	TE-30 g/mlμ	CN-10 μg/ml	ME-5 μg/ml	E-15 μg/ml	CIP-10 μg/ml	P-10 μg/ml
E. coli	I	S	R	R	S	R
P. aeruginosa	I	S	R	R	S	R
P. vulgaris	I	S	R	R	S	R
S. marcescens	I	S	R	R	S	R
A. baumannii	I	S	R	R	S	R
S. aureus	R	S	R	I	R	R
S. pyogenes	S	S	R	S	S	S

I: Intermediate, R: Resistance, S: Sensitive. *E. coli: Escherichia coli, P. aeruginosa: Pseudomonas aeruginosa, P. vulgaris: Proteus vulgaris, A. baumannii: Acinetobacter baumannii, S. aureus: Staphylococcus aureus, S. pyogenes: Streptococcus pyogenes*

Table 2: Antibacterial activity of TiO_2 NPs against some pathogenic bacteria strain (inhibition zone diameter in mm)

Bacterial strain	TiO_2 31.25 μg/ml	TiO_2 62.5 μg/ml	TiO_2 125 μg/ml	TiO_2 250 μg/ml	TiO_2 500 μg/ml
E. coli	6±1.00000	7.3333±0.76376	7±1.00000	12±2.00000	13±1.00000
P. aeruginosa	10±1.0000	11±1.0000	11.3333±1.25831	13±1.0000	14.3333±0.57735
P. vulgaris	5.3333±0.76376	6.1667±1.04083	8.3333±2.51661	9±1.0000	10.3333±0.76376
S. marcescens	11±1.00000	11±2.64575	11.3333±1.52753	13±0.86603	13±1.0000
A. baumannii	14±1.00000	14.50±0.50000	16.3333±1.25831	18±1.00000	20±1.00000
S. aureus	11±1.00000	12±1.00000	13.3333±1.25831	13±1.00000	13.3333±1.04083
S. pyogenes	20±1.00000	23.33±1.04083	24±1.00000	24.33±0.7637	24±1.00000
LSD (0.05)			1.568		

E. coli: Escherichia coli, P. aeruginosa: Pseudomonas aeruginosa, P. vulgaris: Proteus vulgaris, A. baumannii: Acinetobacter baumannii, S. aureus: Staphylococcus aureus, S. pyogenes: Streptococcus pyogenes, TiO_2: Titanium dioxide, NPs: Nanoparticles, LSD: Least significant difference

Table 3: MIC and MBC concentration (mg/ml) of TiO_2 NPs for some pathogenic bacteria

Bacterial strain	TiO_2 NPs MIC	TiO_2 NPs MBC
E. coli	125	500
P. aeruginosa	62.5	250
P. vulgaris	125	500
S. marcescens	62.5	250
A. baumannii	31.25	125
S. aureus	125	500
S. pyogenes	31.25	125

MIC: Minimal inhibitory concentration, MBC: Minimal bactericidal concentration, TiO_2: Titanium dioxide, NPs: Nanoparticles, *E. coli: Escherichia coli, P. aeruginosa: Pseudomonas aeruginosa, P. vulgaris: Proteus vulgaris, A. baumannii: Acinetobacter baumannii, S. aureus: Staphylococcus aureus, S. pyogenes: Streptococcus pyogenes*

Table 4: Antibiofilm activity of minimum inhibitory concentration (MIC value in mg/ml) of TiO_2 NPs towered pathogenic bacterial strain

Bacterial strain	Without TiO_2 NPs	With TiO_2 NPs
E. coli	Strong	Weak
P. aeruginosa	Strong	None
P. vulgaris	Moderate	None
S. marcescens	Strong	None
A. baumannii	Moderate	None
S. aureus	Moderate	Weak
S. pyogenes	Strong	Weak

E. coli: Escherichia coli, P. aeruginosa: Pseudomonas aeruginosa, P. vulgaris: Proteus vulgaris, A. baumannii: Acinetobacter baumannii, S. aureus: Staphylococcus aureus, S. pyogenes: Streptococcus pyogenes, TiO_2: Titanium dioxide, NPs: Nanoparticles, MIC: Minimal inhibitory concentration

frequently less than that of G+ve biofilms, that related to the binding of G–ve bacteriaispoorly withdye than gram positive bacteria. Among all the pathogenic bacteria, G−ve was more susceptible for inhibited their biofilm than G+ve on these surfaces. The attack of TiO_2 NPs on sulfhydryl (thiol) or disulfide groups present in the membrane protein results information of more stable S– TiO_2 bond with groups of –SH; thus, the reaction of inhibiting electron transport chain and enzyme catalyzed that are necessary to the formation of bacterial biofilm.[22]

The inhibition of bacterial biofilm activity occurs during 24 h in G−ve bacteria because to its thinner layer of peptidoglycan in cell wall compared to the G+ve bacteria that mean the inhibition bacterial biofilm increases as a direct relationship with increase the incubation time with exposure to the NPs.[23] Several studies have believed the germicidal mechanisms of TiO_2 nanoparticles involving release of positively charge ions to reaction medium linked to negative charges thiol group (-SH) of the proteins on the cytoplasmic membrane.[24] This reaction leads to capture the cell wall and increased permeability beside it causes deform the structure of cellular components such as DNA, ribosomes, and cellular enzymes, caused oxidization and finally death of microbial cell.[25]

The antibacterial activity is increase with decreasing the particle size of TiO_2 NPs with increasing the specific surface area and dislocation density. Metal NPs have high surface area, which enhances bactericidal activity than the large size NPs; they realize cytotoxicity to the bacteria.[26] Recent study used TiO_2 NPs incorporated or coated packaging of food and tools of food preparing has also interest, Chawenqkijwaich and Hayata[27] concluded that the coated film coated by TiO_2 NPs could inhibit and reduce the bacterial contamination completely or incompletely on the surface of different

food products and the risks of bacterial growth can reduce on many fresh-cut products. Inhibition activity of metallic NPs on biofilm formation of bacteria has been importance, as the device-related infections, especially MDR bacteria that cause of many morbidity and mortality in different hospitalized patients.[28,29] Significant changes in bacterial biofilm after treatment with TiO_2 NPs, NPs caused altered gene expression relating to growth and biofilm formation.[30] TiO_2 NPs lead to larger reduction of bacterial biofilm formation in the glass surface.[31]

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Source of support: Nil; Conflict of interest: None Declared