Titanium Oxide (TiO$_2$) Nanoparticles for Treatment of Wound Infection

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Abstract

Wound infections is one of the major problems worldwide. Millions of people around the world require several medical treatments for wound infections. The extensive use of antibiotics to treat wound infection leads to emerging new microbial strains that are resistant to many antibiotics. There is a growing concern on the emergence and re-emergence of drug-resistant pathogens such as multi-resistant bacterial strains. Hence, the development of new antimicrobial compounds or the modification of those that already exist to improve antibacterial activity is a high research priority. Metallic nanoparticles (NPs) are considered as new alternative treatment for wound infection with superior antibacterial activity. In this study, new formulation of titanium oxide (TiO$_2$) NPs with different sizes were synthesized and characterized. Genotoxicity, mutagenicity and antibacterial activities of TiO$_2$ NPs against the causative agents of wound infection were investigated. Antibacterial activity of TiO$_2$ NPs was conducted against three ATCC® bacterial strains: methicillin-resistant Staphylococcus aureus (MRSA), Escherichia coli and Pseudomonas aeruginosa. The results clearly illustrate a superior antibacterial activity of all newly formulated TiO$_2$ NPs against the most causative agents of wound infection. Most of our TiO$_2$ NPs showed non-genotoxic and non-mutagenic results at the maximum concentrations. Findings of this study will enhance the future of the therapeutic strategies against the resistant pathogenic strains that cause wound infections.

Keywords: TiO$_2$, nanoparticles, antibacterial activity, multi-drug-resistance pathogens, MRSA, E. coli, P. aeruginosa, genotoxicity
INTRODUCTION

The emergence of antimicrobial resistance pathogenic strains considered as one of the major concerns World-Wide (Bassetti et al., 2015). Wound infection accounted as a common life-threatening global health problem resulting in 300,000 death every year (Song et al., 2016). Recent studies confirmed that chronic wound infections affect about 6.5 million people in U.S. alone (Kline and Bowdish, 2016). Delayed wound healing occurs due to several factors such as age, chronic diseases and infection with the pathogenic microorganisms (Morton and Phillips, 2016). These factors increase the spread of infection to the surrounding tissue and longer patients hospitalization (Gainza et al., 2015, Insan et al., 2015). Wounds are vulnerable to be infected with different microorganisms (Singh et al., 2014, Kaushik et al., 2019, Khansa et al., 2019). Thus, the inappropriate use of antibiotics such as β-lactams, vancomycin, daptomycin and rifampicin leads to the development and dissemination of multidrug-resistant (MDR) bacteria (Chudobova et al., 2019, Fried et al., 2015). The most common MDR bacterial species colonize wounds are methicillin-resistance S. aureus (MRSA) followed by E. coli, P. aeruginosa, Enterobacter species, Acinetobacter species, Klebsiella species and Enterococcus species (Fig. 1) (Gupta et al., 2015, Serra et al., 2015, Dhar and Han, 2020). Chowdhury et al., reported that the prevalence of bacteria isolated from infected wounds were 80% Gram negative (mainly E. coli and Pseudomonas) and 20% were Gram positive (MRSA) (Chowdhury et al., 2016, Atef et al., 2019).

Researchers are seeking out for alternative treatment scenarios to overcome the antibiotics resistance crisis since MDR pathogens take over 25,000 lives in the European Union and 23,000 lives in the USA every year (Baym et al., 2016). Nowadays, metallic NPs have been studied as highly promising alternative approach to treat wound infection (Huh and Kwon, 2011, Pachaiappan et al., 2020). These NPs have a potential broad spectrum antimicrobial activity and able to inhibit a wide range of MDR bacteria, including MRSA, P. aeruginosa and E. coli (Yah and Simate, 2015, Mihai et al., 2019). The antimicrobial activity of NPs are driven by several factors such as size, surface charge, shape and concentration (Sportelli et al., 2016). Titanium oxide (TiO_2) NPs have been widely used as photocatalysts among all photocatalytic compounds (Ravishankar Rai and Jamuna Bai, 2011, Naskar and Kim, 2020). TiO_2 NPs are self-cleaning, non-toxic, chemically stable highly photo-reactive and have broad-spectrum antibiotic capability (Priyanka et al., 2016, Bui et al., 2017).

In this study, we investigated the antibacterial activity of newly formulated and synthesized TiO_2 NPs against the most common MDR pathogenic strains that cause wound infections. The antibacterial activity of TiO_2 NPs has been investigated against the tested MDR strains at dose response manner versus several exposure time to determine their best inhibitory effect at a specific time and concentration. Introducing titanium oxide (TiO_2) NPs as antibacterial agents are pushing for a novel step in the development and improvement of revolutionary therapeutics strategies.

MATERIALS AND METHODS

Synthesis and Preparation of TiO_2 Nanoparticles

TiO_2 NPs with different sizes were prepared at the nanotechnology centre, King Abdulaziz University. The synthesis of TiO_2 monocry stalline structures with diameter of 3~8 nm was achieved by hydrothermal and solvothermal conditions by microwave-assisted green synthesis. The electromagnetic energy of microwave has a frequency range between (0.3-300) GHz that equivalent to meV energy from (1.24×10^{-6}) to (1.24×10^{-3}) (Mirzai and Neri, 2016, Yadav et al., 2020). All samples were synthesized using 3.38 mM of Titanium (IV) Isopropoxide (Ti [OCH (CH_3)_2]_4) that dissolved in different ratio of deionized water and ethanol. The mixtures were adjusted to 50 ml in 100 ml Teflon vessel by adding the solvent then sealed it. The synthesis was conducted at 170 ˚C in microwave oven for 90 minutes then cooled down to room temperature. TiO_2 nanopowders were dried in desiccator after centrifugation and washing for three times with deionized water.

Characterization of TiO_2 NPs

X-Ray Diffraction (XRD) analysis was used to determine the particles size and nature. XRD of TiO_2 NPs measurements was performed by using Rigaku, Ultima-IV, which equipped with Cu-Kα
radiation (λ=1.54060 nm) and operated at 40 kV and 40 mA at room temperature. The XRD spectra were measured at step size 0.05°C and 2θ angular region lied between 10°C to 80°C. In addition, the morphological features of the synthesized TiO₂ NPs were examined by field emission scanning electron microscope Jeol, Japan (FESEM- JSM-7600F).

**Growth Characterization of Multi-Drug-Resistance Pathogens**

Methicillin-resistant *Staphylococcus aureus* (MRSA) (ATCC® 43300MINIPACK™), *Pseudomonas aeruginosa* (ATCC® 27853™) and *Escherichia coli* (ATCC® 25922™) were purchased from the American Type Culture Collection (ATCC) org. (Manassas, USA). The bacterial strains were characterized by monitoring the optical density (OD) and colony forming units (CFUs) of the bacterial cells over time. Luria-Bertani (LB) agar and broth were purchased from Micromaster Laboratories Pvt. Ltd. (Maharashtra, India) and prepared according to manufacture instructions. A full loop of the overnight second sub-culture colonies of each strain were inoculated in Erlenmeyer flask containing 50 ml LB broth. The inoculum was incubated in a shaker incubator (GFL Shaking Water Bath 1083 from UNIQUE Medical Laboratory Equipment Trading & Services, Sharjah, UAE) at 37°C and 150 rpm for 18 hours. The bacterial growth was monitored by measuring the optical density at wavelength 600 nm (OD$_{600}$) using a spectrophotometer (GENESYS™ 20 Visible Spectrophotometer from Thermo Fisher Scientific Inc., Madison, USA) and CFUs/ml. The bacterial cells were transferred into 50 ml polypropylene conical VWR® high-performance centrifuge tubes with plug caps (VWR International, LLC Radnor, PA, USA) and harvested by centrifugation at 5000 rpm.

The bacterial pellets were washed three times with 10 ml of 0.9% NaCl normal saline and centrifuged at 5000 rpm for 7 min at 25°C. After the third wash, the microbial pellets were re-suspended in 10 ml of 0.9% NaCl normal saline and the CFUs/ml and OD$_{600}$ were measured to determine the optimal growth of viable cells before adding the TiO₂ NPs.

**Antibacterial Activity of TiO₂ Nanoparticles**

Five doses of the synthesized TiO₂ NPs (100, 200, 400, 600 and 800 μg) were used and sterilized by UV light for 45 min. Bacterial growth was monitored using drop-plating method for counting the CFUs/ml (Miles et al., 1938). Each concentration of every NPs sample was dissolved in 5 ml of bacterial suspension and mixed gently by vortex. Serial dilutions (1:10) were accomplished for the five concentrations of TiO₂ NPs by adding 100 μL of the bacterial cells to 900 μL 0.9% NaCl normal saline. Three 10 μL aliquots of the proper dilution were plated onto LB agar plate and incubated overnight at 37°C. Samples were incubated at 37°C shaker incubator with 150 rpm at different time intervals (60, 120 and 150 min).

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**Fig. 1.** The Most Common Causative Agent of Wound Infection. Adopted from (Gupta et al., 2015).
The schematic diagram for the whole experimental protocol of antibacterial activity of the synthesized TiO$_2$ NPs was illustrated in (Fig. 2).

**Monitoring the Growth Curve of the Bacterial Strains Exposed to TiO$_2$ Nanoparticles**

TiO$_2$ NPs were added at different doses (100, 200, 400, 600, and 800 µg/ml) to measure their effect on the growth of bacterial cells. This was handled by processing the effect of presenting the TiO$_2$ NPs on the viable bacterial cells at different time intervals. Drop-plate method was implemented for the recovered samples by spotting 10 µL aliquots in triplicates on LB agar and incubated at 37°C overnight. The dose-response curve experiment was completed after (150 min) when the bacterial cells reached to the decline phase. The antibacterial activity of TiO$_2$ NPs against tested bacterial strains was evaluated in dose-response manner (using different concentrations) by counting the CFUs/ml versus time to detect the minimum inhibitory concentration (MIC). A comparison was done from the plotting dose-response curves of CFUs/ml versus time (min) to investigate the ideal TiO$_2$ NPs concentration that exhibited antibacterial effect at a certain exposure time.

**The Mutagenicity and Toxicity Assessment of TiO$_2$ Nanoparticles**

The genotoxicity of TiO$_2$ NPs was conducted using an analytical Genotoxicity SOS-Chromo Test™ Kit purchased from EBPI (Environmental Bio-Detection Products Inc., Mississauga, Ontario, Canada). It is an enzymatic colorimetric assay to detect DNA damaging agents after incubating the tested TiO$_2$ NPs samples with a genetically engineered bacterium *E. coli* PQ37 (Jabbour et al., 2016). The test was performed to detect the genotoxic samples using β-galactosidase (β-gal) and alkaline phosphatase (AP) as a signal of SOS response activation. The amount of β-gal induction is revealing the level of SOS induction and bacterial genotoxicity whereas the AP activity was used to detect the range of bacterial cytotoxicity (Kocak, 2015). Rat liver S-9 fraction was simulated the liver function metabolism for measuring the mutagenic potential of any chemical substances such as TiO$_2$ NPs. The lyophilized bacteria were resuscitated by transferring 10 ml of growth media to the dried bacteria and roughly mixed for 30 seconds. Then, 100 µl from bacterial suspension was transferred to a new bacterial growth medium and incubated.
overnight for (8 - 12) hours in a rotary shaker at 150 rpm at 37°C. The overnight bacterial inoculum was diluted with fresh growth medium using the equation.1 to a final OD$_{600}$ of 0.05. Various concentrations (100, 200, 400, 600 and 800 μg) of each sample of TiO$_2$ NPs were dissolved in 1 ml of 50% dimethyl-sulfoxide (DMSO). The SOS – Chromo Test was performed with and without metabolic activation S-9.

The Required Volume of Culture

\[
\text{Equation 1. The Required Volume for Bacterial Dilution.}
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The first and seventh columns of the 96 - wells microplate contained the six, two-fold dilutions of the positive control, 4-Nitro-Quinoline-N-Oxide (4-NQO) and S-9 positive control, 2-Amino - Anthracene (2-AA), in 10% DMSO. The last raw in the plate was used as negative controls while serial dilution was performed for positive controls. Other columns contained 10 μL aliquots of 10% DMSO and TiO$_2$ NPs in a dose-response manner for each sample without performing serial dilutions. The experimental design of SOS – Chromo Test was illustrated in Fig. 3.

**Statistical Analysis**

The experiments were achieved in triplicate for each strain. All statistical analysis was carried out using Minitab® Statistics software for Windows, version 17.3.1 (Minitab, Inc. USA). The prediction of antibacterial activity of different concentrations of TiO$_2$ NPs to show a reduction in the cell growth (CFUs/ml) were accomplished

| TiO$_2$ NPs Categories | Samples | Size (nm) | Water: Ethanol | Concen. Ti[OCH(CH$_3$)$_2$]$_4$ (mmol) |
|------------------------|---------|-----------|----------------|-------------------------------------|
| Large size More than (> 5 nm) | TiO$_2$ 8 | 7.6 | (25: 75) % | 3.38 |
| | TiO$_2$ 9 | 6 | (50: 50) % | 3.38 |
| Medium Size Between (4 - 5 nm) | TiO$_2$ 12 | 4.6 | (75: 25) % | 3.38 |
| | TiO$_2$ 13 | 4.9 | (100: 0) % | 3.38 |
| Small Size Less (< 5 nm) | TiO$_2$ 10 | 3.4 | (0: 100) % | 3.38 |
| | TiO$_2$ 14 | 3.7 | (100: 0) % | 1.96 |
by analyzing the data points at several period intervals of all experiments for each strain. The non-parametric test Kruskal-Wallis and the T-test analysis were conducted to evaluate statistically significant differences ($p < 0.05$). A 95% confidence level was used for all statistical analysis and the curves were plotted using Excel 2010 for Windows.

**RESULTS**

**Nanoparticles Structure Investigation**

The X-ray diffractometer was used to analyze the crystalline nature, the size and the shape of atoms structure in the sample (Garino et al., 2014). The spectra of TiO$_2$ NPs in Fig. 4 showed multiple crystalline shapes that located at several positions in the sample. The size of TiO$_2$ NPS was calculated by using Scherrer formula (Equation 2), which constant (K) for spherical shape equals (0.9).

\[
D = \frac{K\lambda}{\beta \cos\theta}
\]

Equation 2. The Required Volume for Bacterial Dilution.

The broad peaks illustrated relatively small size particles whereas their intensities varied among the several TiO$_2$ NPs. Phases (101), (103), (200), (105), (213), (116) and (107) were parallel to the diffraction peaks displayed at 2\(\theta\) of 25.3, 37.8, 47.8, 54.1, 62.6, 70.4 and 75.5, respectively. The calculated samples size was found to be exceeded the excitation Bohrer diameter by about 1.5 nm. Obviously, the reported nano-crystallite sizes revealed that sample TiO$_2$ 10 has the smallest size (3.4 nm) compared to other TiO$_2$ NPs mentioned in Table 1.

**Nanoparticles Morphological Features**

Surface morphology, topography and chemical composition of the synthesized TiO$_2$ (8-14) had been recorded using field emission scanning electron microscope (FESEM- JSM-7600F). The examined nanoparticles powder deposited on carbon tripe and demonstrated high 3-dimensional resolution images of materials. However, the images exposed condensed
aggregated spherical like clusters of TiO$_2$ NPs with approximate average sizes between 3-15 nm. Sample TiO$_2$ 10 had the smallest particle size as shown in Fig. 5 while other sample’s sizes varied between medium to large size. Thus, this variation in the particle sizes might be correlated to different ratio of solvents and deionized water used in the synthesis of the TiO$_2$ NPs.

**Antibacterial activity of TiO$_2$ nanoparticles with particle size more than 5 nm**

The results of antibacterial activity for TiO$_2$ 8 and TiO$_2$ 9 NPs against MRSA, *P. aeruginosa* and *E. coli* are illustrated in Fig. 6. There was a significant reduction in the number of CFUs for MRSA exposed to both TiO$_2$ 8 and TiO$_2$ 9 NPs at the lower (100 µg/ml) and the highest concentrations (800 µg/ml) after 150 min of exposure time ($p = 0.018$ and 0.016, respectively). Similarly, TiO$_2$ 8 NPs confirmed a significant antibacterial activity against *E. coli* at the maximum concentration (800 µg/ml) only after 60 min of exposure time ($p = 0.034$) while TiO$_2$ 9 NPs showed a significant antibacterial activity with the lowest (100 µg/ml) and the highest (800 µg/ml) after 150 min ($p = 0.028$). There was a significant reduction in the number of CFUs/ml of *P. aeruginosa* exposed to 100 µg/ml TiO$_2$ 8 NPs after 60 min of exposure time ($p = 0.028$). Similarly, TiO$_2$ 10 NPs have illustrated superior antibacterial activity against MRSA *P. aeruginosa* and *E. coli* at the lowest (100 µg/ml) and the highest (800 µg/ml) concentrations after only 60 min exposure time ($p = 0.001$ and 0.034, respectively).

**Antibacterial activity of TiO$_2$ nanoparticles with particle size between 4 – 5 nm**

The antibacterial activity results for TiO$_2$ 12 and TiO$_2$ 13 NPs with particles size between 4-5 nm against MRSA, *E. coli* and *P. aeruginosa* were presented in Fig. 7. The antibacterial activity of TiO$_2$ NPs was monitored in a dose-response curve using different concentrations of the NPs (100, 200, 400, 600 and 800 µg/ml). TiO$_2$ 12 NPs had superior antibacterial activity against all of three bacterial strains (Fig. 7). The maximum concentration (800 µg/ml) was more effective against MRSA and *E. coli* after 120 min of exposure time. The effect of NPs significantly increased after 150 min of exposure time ($p = 0.009$ and 0.019, respectively). There was a significant reduction in the number of CFUs/ml of *P. aeruginosa* after exposure to 100 µg/ml TiO$_2$ 12 NPs ($p = 0.028$). Similarly, TiO$_2$ 13 NPs have illustrated superior antibacterial activity against MRSA *P. aeruginosa* and *E. coli* at the lowest (100 µg/ml) and the highest (800 µg/ml) concentrations after only 60 min exposure time ($p = 0.001$ and 0.034, respectively).

**Antibacterial activity of TiO$_2$ nanoparticles with particle size less than 5 nm**

The antibacterial activities of TiO$_2$ 10 and TiO$_2$ 14 NPs with particles size less than 5 nm were illustrated in Fig. 8. There was a significant decrease in the number of CFUs/ml of MRSA exposed to different concentrations (100 µg/ml...
ml – 800 µg/ml) of TiO$_2$ 10 NPs only after 60 min exposure time ($p = 0.002$ and 0.006, respectively). Similar observation was reported for TiO$_2$ 10 NPs tested against E. coli. Nevertheless, TiO$_2$ 10 NPs demonstrated limited antibacterial activity against P. aeruginosa (Fig. 8).

TiO$_2$ 14 NPs displayed a remarkable antibacterial activity against MRSA and E. coli only after 60 min of exposure time with all of the tested concentrations: 100, 200, 400, 600 and 800 µg/ml (Fig. 8). Nevertheless, the effect was limited with P. aeruginosa as only 800 µg/ml of TiO$_2$ 14 NPs showed antibacterial activity after 150 min of exposure time ($p = 0.020$) (Fig. 8).

**Mutagenicity and Toxicity Assessment of TiO$_2$ NPs**

The mutagenicity and genotoxicity results were assessed by calculating the SOS - Induction Factor (SOSIF) (Equation.3). The calculated optical density of all TiO$_2$ NPs in the absence and the presence of S-9 activation enzyme were classified according to SOSIF classification (Table 2). Most concentrations (100, 200, 400, 600 and 800 µg/ml) of the synthesized TiO$_2$ NPs were non-genotoxic and non-mutagenic (Fig. 9). However, concentrations reported inconclusive, require more investigations to figure out their toxicity (Table 3).

![Fig.6. Antibacterial Activity of TiO$_2$ Nanoparticles with particle size more than 5 nm against MRSA, P. aeruginosa and E. coli.](image-url)
**DISCUSSION**

The emergence of antimicrobial resistance pathogenic strains considered as one of the major concerns World-Wide for human health and dramatically raised economic costs. MRSA, *P. aeruginosa* and *E. coli* are highly resistant to broad-spectrum of antibiotics which considered as the most causative agents of nosocomial infections. These strains become an endemic in hospitals and long-term care facilities because they show a dramatic increase in resistance to antimicrobial agents, especially vancomycin (John et al., 2015). Multi-drug-resistance (MDR) bacteria are tremendously hard to eradicate and guide researchers towards discovering novel strategies for treatment of wound infection. Therefore, introducing new antimicrobial agents can control the rate of morbidity and mortality that result from infectious diseases such as wound infections. Metallic NPs have been studied as highly promising alternative approach to treat wound infection...
(Huh and Kwon, 2011, Pachaiappan et al., 2020). TiO₂ NPs are inexpensive, biologically and chemically stable, and corrosion-resistant (Xiao et al., 2015). Nowadays, the field of materials science consider TiO₂ as an eco-friendly material and promising semiconductor with antimicrobial activity (Gopinath et al., 2016, Periyat et al., 2016).

In this study, the antibacterial activity of different concentrations and sizes of the synthesized anatase TiO₂ nanoparticles (NPs) was investigated against MDR strains. Our findings showed that all samples of TiO₂ NPs possessed antibacterial activity against the tested strains. Nevertheless, TiO₂ 12 (4.6 nm) and TiO₂ 13 (4.9 nm) with medium size had the best antibacterial activity against all the three strains at the minimum concentration (100 µg/ml). These findings were in agreement with previous study of the antibacterial activity of metallic oxide NPs (Alkaim, 2017, Dadi Table 2. The SOSIF Classification

| SOSIF    | Interpretation       | Comments               |
|----------|----------------------|------------------------|
| < 1.5    | Non-Genotoxic        | Safe to be used        |
| 1.5 - 2.0| Inconclusive         | Required further       |
| > 2.0    | Genotoxic            | Required more          |

Fig. 8. Antibacterial Activity of TiO₂ Nanoparticles with particle size less than 5 nm against MRSA, P. aeruginosa and E. coli.
et al., 2019, Kaushik et al., 2019). Antibacterial activity of TiO₂ NPs is very complicated and several factors such as NPs physicochemical properties might affect their activity (Hajipour et al., 2012). The exact mechanisms of bacterial cell inhibition or death due to NPs effect were completely unclear and not fully understood. Many studies conducted to investigate the exact mechanisms of bacterial cell inhibition or death and suggested several possible scenarios. The possible inhibitory mechanism can be through electrostatic interaction and oxidative stress. Fig. 10 summarizes all possible antibacterial mechanisms of TiO₂ NPs against bacterial strains. The physicochemical properties of TiO₂ NPs play a role in their antibacterial activity against bacterial community as reported in several studies (Zhao et al., 2010, Jesline et al., 2015, Hoseinzadeh et al., 2017, Kumar et al., 2017a). Many factors

![Fig. 9. SOS - Chromo Test Results](image)

![Fig. 10. Overview of Possible Antibacterial Activity Mechanisms for TiO₂ NPs](image)
influenced the bacterial cell death mechanism of NPs included size, shape, concentration, electrical charge, surface structure, solvents and the exposure time (Sirelkhatim et al., 2015). Moreover, different ratio of the solvents and the concentrations of titanium used to synthesize TiO$_2$ NPs may influence their antibacterial activity as reported in previous studies (Hu et al., 2012). Several studies showed the effect of using different solvents, precursor concentrations and conditions on the size, shape, crystal distribution, surface properties and antibacterial activity of NPs (Kumar et al., 2017b). The mixture percentage of deionized water (H$_2$O) to Ethanol (CH$_3$CH$_2$OH or C$_2$H$_6$O) and concentrations of Titanium (IV) Isopropoxide (Ti[OCH(CH$_3$)$_2$]$_4$) in each NPs sample was illustrated in Table 1.

The nanoparticles with large size (> 5 nm) such as TiO$_2$ 8 (7.6 nm) and TiO$_2$ 9 (6 nm) were prepared using half or less percentage of water to ethanol. These NPs illustrated more antibacterial activity against MRSA and *E. coli* with limited activity against *P. aeruginosa*. Samples with small size (< 5 nm) such as TiO$_2$ 10 (3.4 nm) was synthesized using ethanol only as a solvent had greater antibacterial activity against MRSA and *E. coli* and least effect against *P. aeruginosa*. Small size, fine shape and narrow distribution of particles are correlated with low precursor concentration such as sample TiO$_2$ 14 (3.7 nm) that prepared using only water as solvent and half concentration of titanium precursor (1.96 mmol). This sample had superior antibacterial activity against all of the three bacterial strains (Hu et al., 2012). On the other hand, the medium sized NPs between (4 - 5 nm) were prepared with high percentage of water to ethanol such as TiO$_2$ 12 (4.6 nm) and TiO$_2$ 13 (4.9 nm) have shown a significant antibacterial activity against MRSA, *E. coli* and *P. aeruginosa* at all concentrations. Antibacterial activity limitation towards *P. aeruginosa* could be correlated to the nature of resistance mechanism which is multi-factorial (Chatterjee et al., 2016). This strain possessed an intrinsic resistance and able to develop a resistance readily and rapidly resulting in decreased membrane permeability 12-100-fold than other bacteria (Taylor et al., 2014, Ramirez-Estrada et al., 2016).

Many studies reported that nearly most of the TiO$_2$ NPs are non-genotoxic/mutagenic (Chen et al., 2014). Most concentrations of our synthesized TiO$_2$ NPs showed non-genotoxic and non-mutagenic effect at the maximum concentration (800 µg/ml). Though, some concentrations of our particles displayed genotoxic effect and this could be due to the solvent used for dissolving the NPs which was 50% dimethyl-sulfoxide (DMSO), and 2% of it considered toxic for the cells (Alhadrami and Paton, 2013).

**Table 3.** The Results of SOSIF Genotoxicity / Mutagenicity (orange colour is Non-Genotoxic/Mutagenic, yellow colour represents Genotoxicity/Mutagenicity, and white colour indicates inconclusive results)

| Genotoxicity without S-9 | TiO$_2$ | TiO$_2$ | TiO$_2$ | TiO$_2$ | TiO$_2$ | TiO$_2$ | TiO$_2$ | TiO$_2$ | PC |
|--------------------------|--------|--------|--------|--------|--------|--------|--------|--------|----|
| Conc 100                 | 1.8994 | 1.7999 | 1.5802 | 0.9707 | 1.8676 | 1.5846 | 1.7868 | 16.9241 |
| Conc 200                 | 1.3607 | 1.6037 | 1.3076 | 0.9081 | 1.5920 | 1.4195 | 1.3145 | 21.2512 |
| Conc 400                 | 1.3513 | 1.7251 | 1.4201 | 1.4125 | 1.8510 | 2.0910 | 1.5588 | 14.3697 |
| Conc 600                 | 1.8405 | 1.9986 | 1.5881 | 2.2077 | 1.7622 | 2.0802 | 2.0140 | 12.4203 |
| Conc 800                 | 1.7961 | 1.4087 | 1.5017 | 1.2873 | 1.3918 | 1.3063 | 2.0611 | 8.39894 |

| Genotoxicity with S-9 | TiO$_2$ | TiO$_2$ | TiO$_2$ | TiO$_2$ | TiO$_2$ | TiO$_2$ | TiO$_2$ | TiO$_2$ | S-9 (PC) |
|-----------------------|--------|--------|--------|--------|--------|--------|--------|--------|----------|
| Conc 100              | 1.1021 | 1.1589 | 1.1156 | 1.0490 | 1.1013 | 1.7516 | 1.2757 | 3.3862 |
| Conc 200              | 1.3400 | 1.3729 | 1.2264 | 1.2167 | 1.3721 | 2.9208 | 2.3920 | 2.3401 |
| Conc 400              | 1.1579 | 1.3046 | 1.1121 | 1.3565 | 1.0876 | 2.4663 | 1.4064 | 1.9137 |
| Conc 600              | 0.8274 | 1.1880 | 1.0468 | 1.2136 | 1.3445 | 1.5724 | 1.3003 | 1.3977 |
| Conc 800              | 1.2126 | 1.1005 | 1.1221 | 1.1128 | 1.1574 | 1.9634 | 1.5876 | 1.2226 |

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CONCLUSION AND FUTURE PERSPECTIVE
Nowadays, there is a great competition in finding novel technologies against MDR bacteria. Nanoparticles are widely used as antibacterial agents against several MDR pathogens. Thus, titanium oxide NPs can be a proper alternative antibacterial agent. This study sheds light on the antibacterial activity of TiO$_2$ NPs on MDR microorganisms that cause wound infections. The TiO$_2$ NPs exhibited high efficacy as a strong antibacterial agent towards the tested strains. Their antibacterial activity against MDR pathogens was as follows: MRSA (Gram-positive) > E. coli (Gram-negative) > P. aeruginosa (Gram-negative). Thus, the most effective samples that demonstrated superior antibacterial activity is ranked as TiO$_2$ 12 > TiO$_2$ 13 > TiO$_2$ 14 > TiO$_2$ 10 > TiO$_2$ 9 ≥ TiO$_2$ 8. The synthesized TiO$_2$ NPs were non-genotoxic/mutagenic. Thus, these NPs can be great alternative to antibiotics for the treatment of wound infection. This demonstrates potential applications of these NPs in medical and biomedical fields.

ACKNOWLEDGMENTS
The authors acknowledge with thanks DSR for technical and financial support.

CONFLICT OF INTEREST
The authors declare that there is no conflict of interest.

AUTHORS’ CONTRIBUTION
Both authors have been equally contributed towards the experimental work and writing the manuscript.

FUNDING
This project was funded by Deanship of Scientific Research (DSR), King Abdulaziz University, Jeddah, Saudi Arabia, under grant G-1439/142/248.

DATA AVAILABILITY
All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT
This article does not contain any studies with human participants or animals performed by any of the authors.

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