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Titanium Oxide (TiO_2) Nanoparticles for Treatment of Wound Infection

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Abstract

Wound infections evidently appeared in times of World War I that accounted a significant mortality and morbidity rate among injured soldiers. Currently, around 11 million people worldwide require medical treatment for wound infections and 300,000 die every year due to untreated wound infection. 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 priority area of research. During the past few decades, nanotechnology has arisen with new promising technology for synthesis of nanobiomaterials. Metallic nanoparticles (NPs) are considered as new alternative treatment 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.*

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1. 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 and Insan *et al*., 2015). Wounds are vulnerable to be infected with different microorganisms (Singh *et al*., 2014). Thus, the inappropriate use of antibiotics such as β-lactams, vancomycin, daptomycin and rifampicin leads to the development and dissemination of multi-drug-resistant (MDR) bacteria (Chudobova *et al*., 2015 and Friães *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* (Figure. 1) (Gupta *et al*., 2015 and Serra *et al*., 2015). 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).

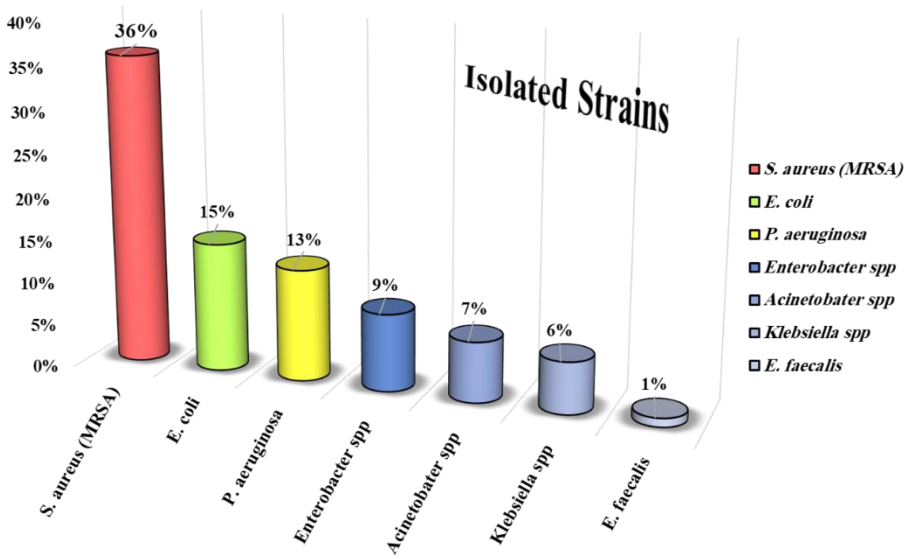

Figure.1. The Most Common Causative Agent of Wound Infection. Adopted from (Gupta *et al*., 2015).

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

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alternative approach to treat wound infection (Huh and Kwon, 2011). 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). 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). TiO_2 NPs are self-cleaning, non-toxic, chemically stable highly photo-reactive and have broad-spectrum antibiotic capability (Priyanka *et al*., 2016).

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.

2. Materials and Methods

2.1 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 monocrystalline structures with diameter of 3~8 nm was achieved by hydrothermal and solvothermal conditions. All samples were synthesized using 3.38 mM of Titanium (IV) Isopropoxide (Ti [OCH $(CH_3)_2]_4$). Samples were dissolved in deionized water and ethanol at 170℃ for 90 minutes. Particles size was analysed and determined using X-Ray Diffraction (XRD).

2.2 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 ℃ and 150 rpm for 18 hours. The bacterial growth was monitored by measuring the optical density at wavelength 600 nm (OD_{600}) using a

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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 TiO_2 NPs.

2.3 Antibacterial Activity of TiO_2 Nanoparticles

Five doses of the synthesized TiO_2 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 TiO_2 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). The schematic diagram for the whole experimental protocol of antibacterial activity of the synthesized TiO_2 NPs was illustrated in (Figure.2).

2.4 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 .

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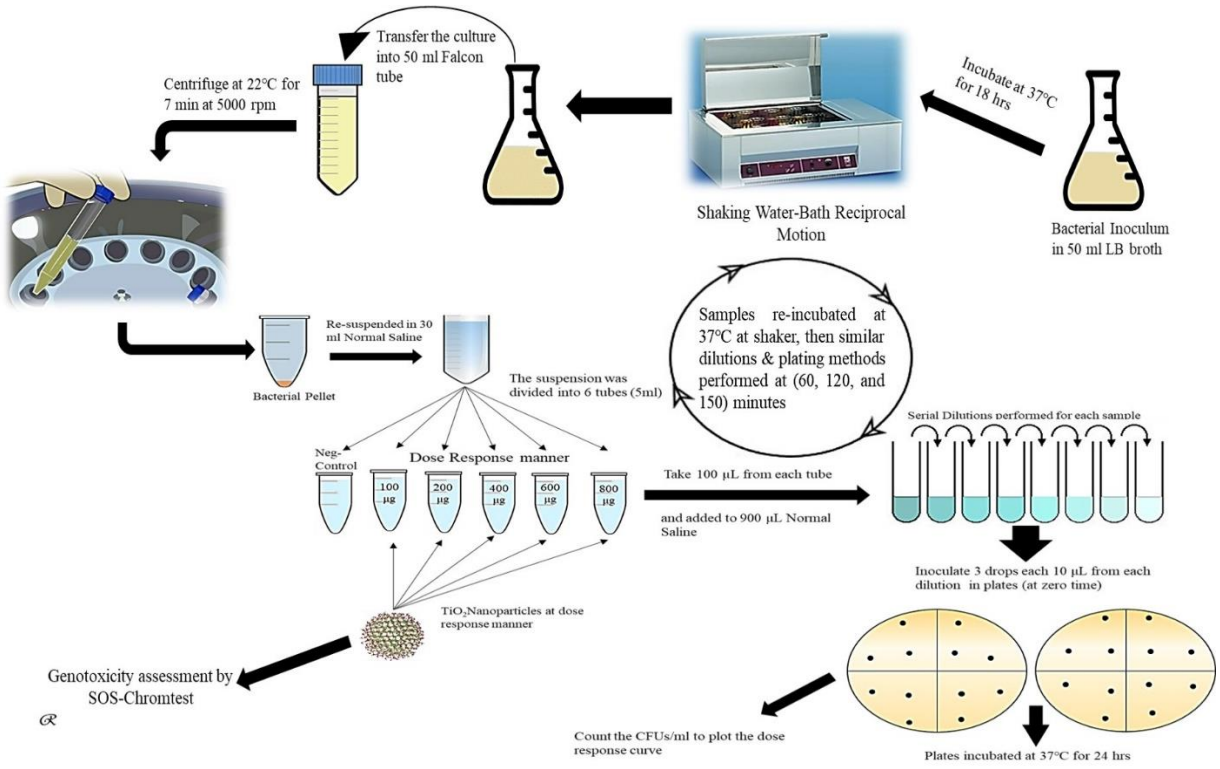

Figure.2. Experimental Design of TiO_2 NPs Antibacterial Activity

2.5 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 incubation 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 is 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 mixed by inverted 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.

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$$\textbf{The Required Volume of Culture} = \frac{\mathbf{0.5}}{\text{OD of overnight culture}}$$

Equation.1. The Required Volume for Bacterial Dilution.

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 Figure. 3.

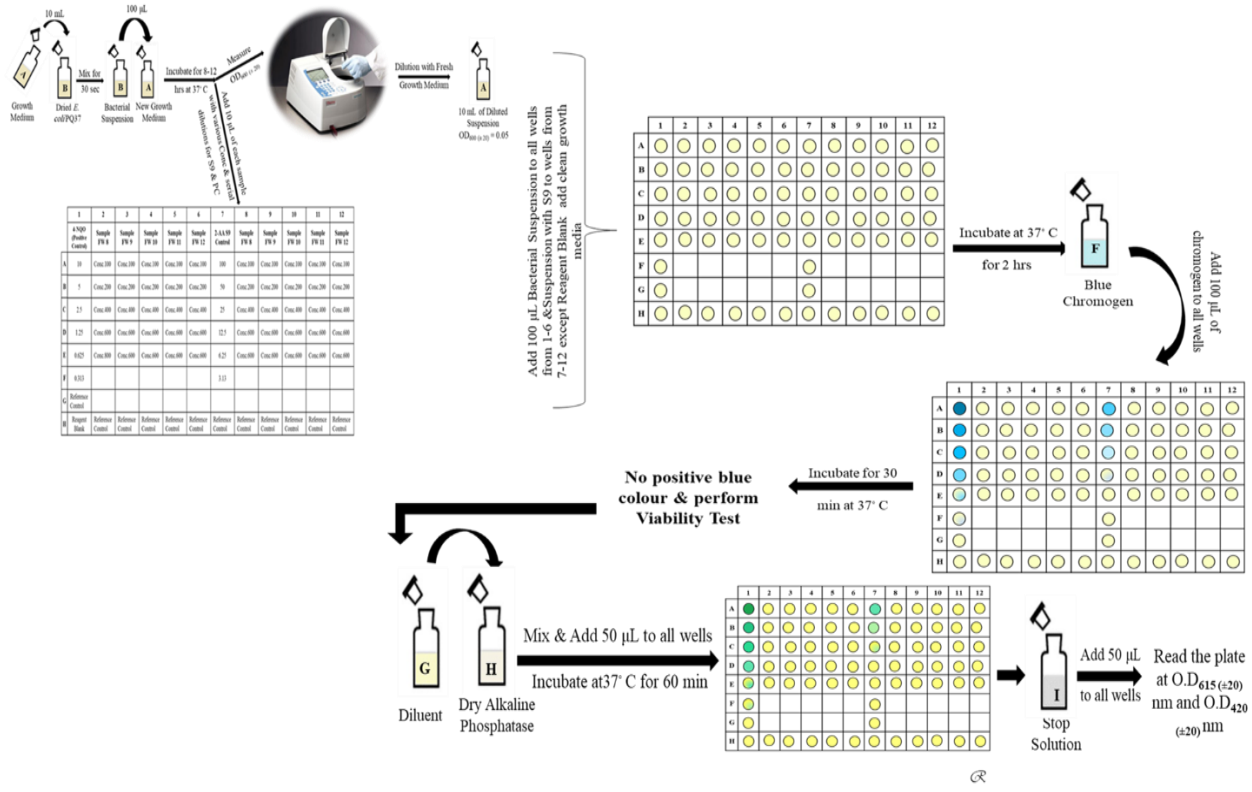

Figure.3. Genotoxicity / Mutagenicity Experimental Design

2.6 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 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 %

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confidence level was used for all statistical analysis and the curves were plotted using Excel 2010 for Windows.

3. Results

3.1 Characterization of TiO_2 NPs

X-Ray Diffraction (XRD) analysis was used to determine the particles size by applying Scherrer's formula. Table.1 illustrates TiO_2 NPs categories, size, solvents percentage and titanium precursor concentration.

Table.1. TiO_2 NPs Categories, Size, Solvents Percentage and Titanium Precursor Concentration.

TiO_2 NPs Categories	Samples	Size (nm)	Water: Ethanol	Concentration of $Ti[OCH(CH_3)_2]_4$ (mmol)
Large size (> 5 nm)	**TiO_2 8**	7.6	(25: 75) %	3.38
	TiO_2 9	6	(50: 50) %	3.38
Medium size (4 - 5 nm)	**TiO_2 12**	4.6	(75: 25) %	3.38
	TiO_2 13	4.9	(100: 0) %	3.38
Small size (< 5 nm)	**TiO_2 10**	3.4	(0: 100) %	3.38
	TiO_2 14	3.7	(100: 0) %	1.96

3.2 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 Figure 4. 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 800 µg/ml TiO_2 8 NPs after 150 min (p = 0.043). Similarly, 100 µg/ml TiO_2 9 NPs caused a significant reduction in the number of CFUs/ml of *P. aeruginosa* after 60 min of exposure time.

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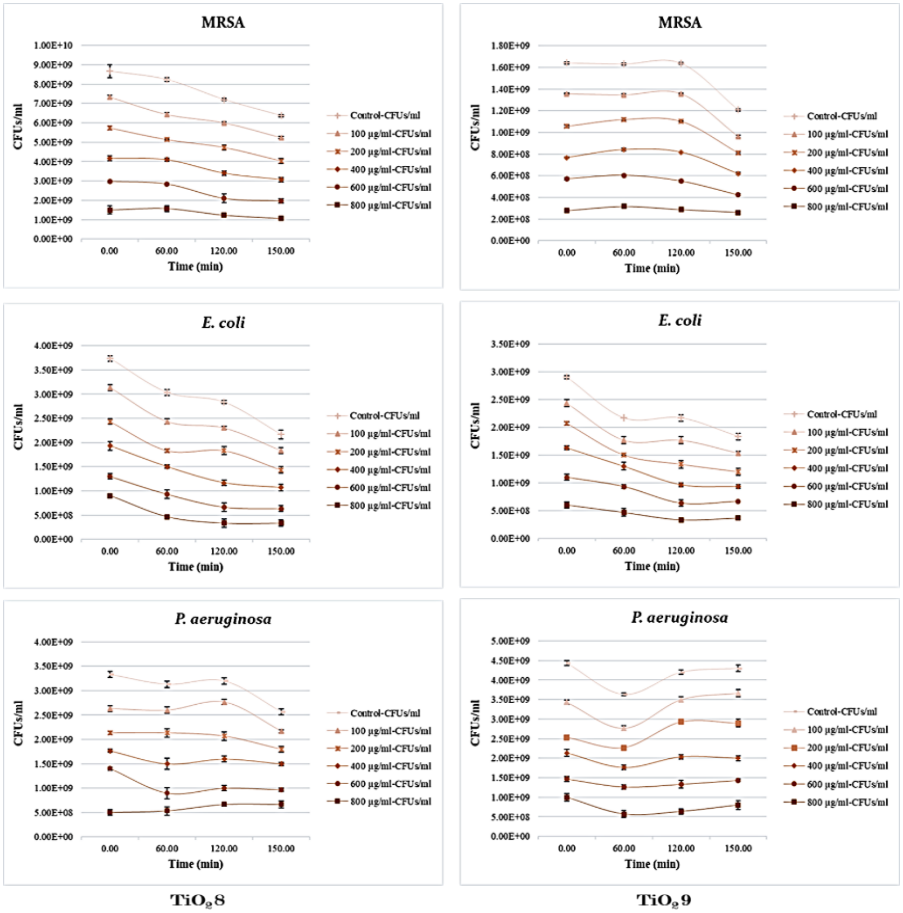

Figure.4. Antibacterial Activity **TiO_2 Nanoparticles with particle size more than 5 nm** against MRSA, *P. aeruginosa* and *E. coli*.

3.3 Antibacterial activity of TiO_2 nanoparticles with particle size less 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 Figure.5. 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 (Figure 5). 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 (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 for 150 min (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).

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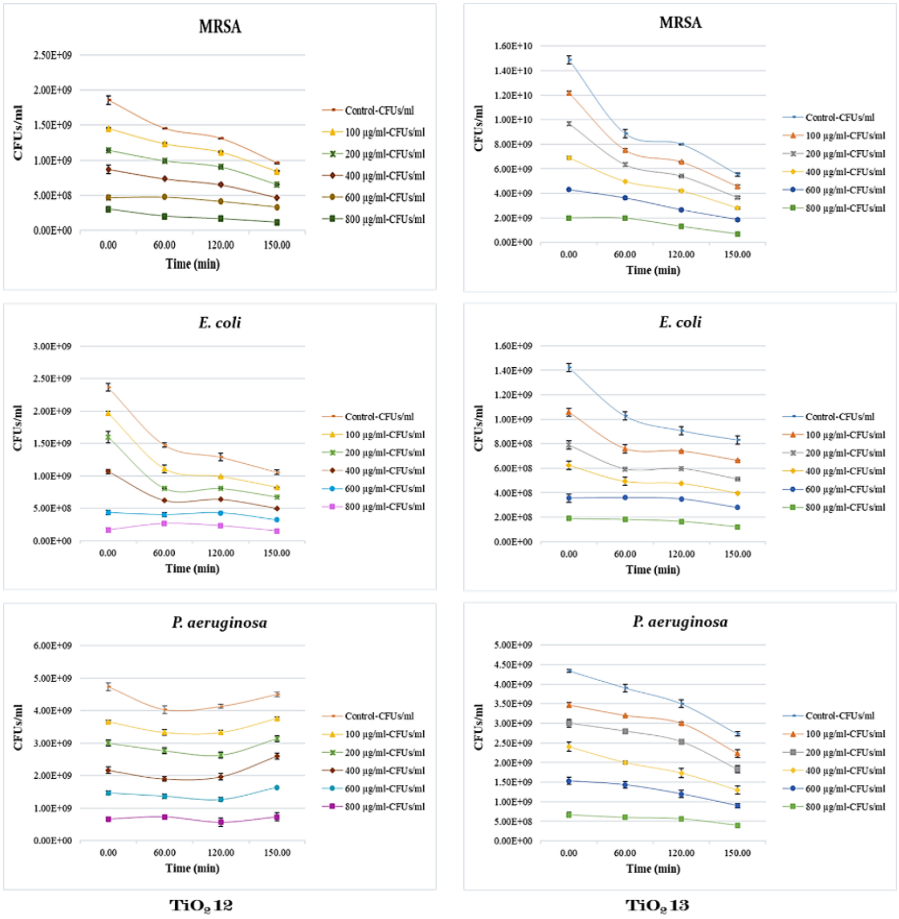

Figure.5. Antibacterial Activity **TiO_2 Nanoparticles with particle size between 4-5 nm** against MRSA, *P. aeruginosa* and *E. coli*.

3.4 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 are illustrated in Figure.6. There was a significant decrease in the number of CFUs/ml of MRSA exposed to different concentrations (100 µg/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* (Figure 6).

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 (Figure 6). Nevertheless, the effect was limited with *P. aeruginosa* as only 800 µg/ml of TiO_2 14 NPs showed antibacterial activity after 150 min (p = 0.020) (Figure 6).

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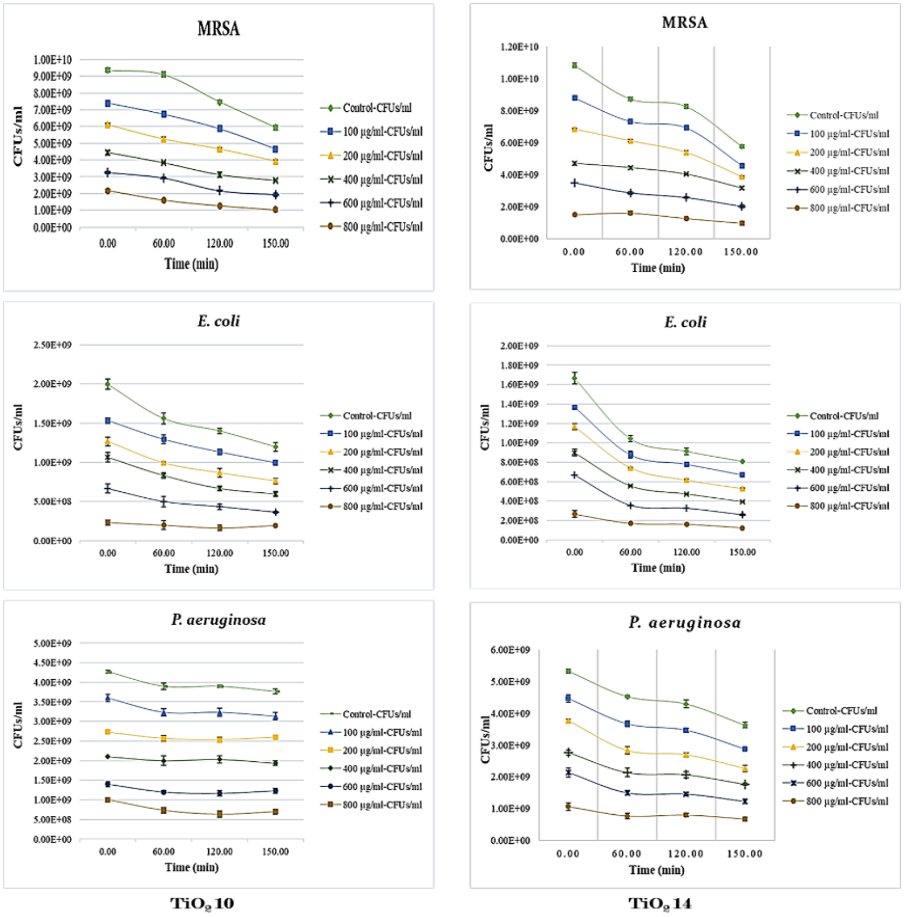

Figure.6. Antibacterial Activity **TiO_2 Nanoparticles with particle size less than 5 nm** against MRSA, *P. aeruginosa* and *E. coli*.

3.5 Mutagenicity and Toxicity Assessment Tests of TiO_2 NPs

The mutagenicity and genotoxicity results were assessed by calculating the SOS - Induction Factor (SOSIF) (Equation.2). 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 (Figure.7). However, concentrations reported inconclusive, genotoxic and mutagenic results will require more investigations to figure out their toxicity (Table.3).

$$\mathbf{SOSIF} = \frac{(OD_{630}\,i) \div (OD_{405}\,i)}{(OD_{630}\,NC) \div (OD_{405}\,NC)}$$

Equation.2. Calculation of SOS - Induction Factor.

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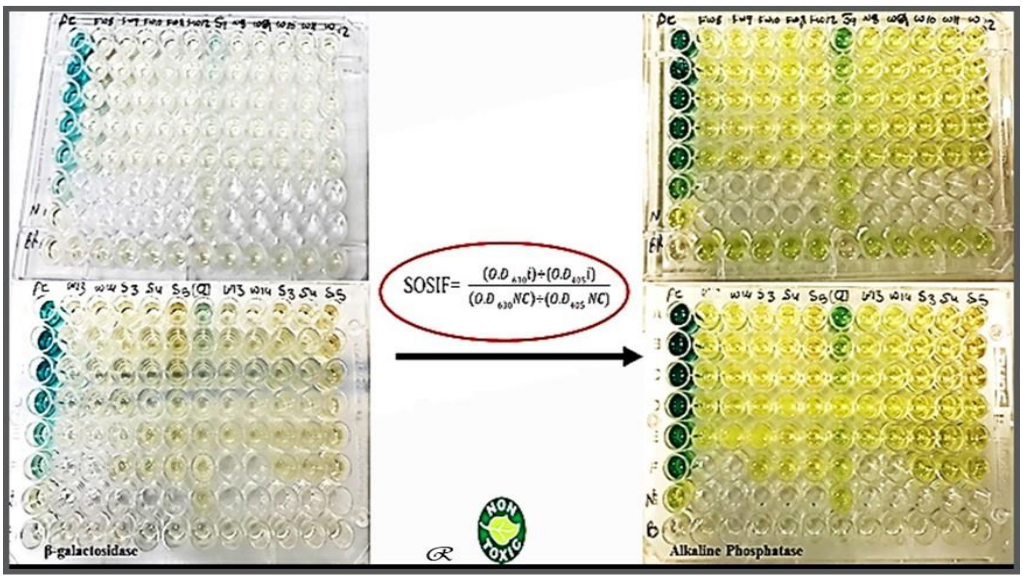

Figure.7. SOS - Chromo Test Results

Table.2. The SOSIF Classification.

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

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

SOS-Induction Factor (SOSIF)

Bacterial Suspension WITHOUT S-9

	$TiO_2$8	$TiO_2$9	$TiO_2$10	$TiO_2$12	$TiO_2$13	$TiO_2$14	PC
Conc 100	1.8994	1.79998	1.58027	1.86769	1.85486	1.78688	**16.9241**
Conc 200	1.36073	1.60371	1.30767	1.59203	1.41954	1.31452	**21.2512**
Conc 400	1.35139	1.72513	1.42012	1.85806	2.09103	1.55882	**14.3697**
Conc 600	1.84057	1.99856	1.58817	1.76223	2.08029	2.01408	**12.4203**
Conc 800	1.79617	1.40874	1.5017	1.39187	1.30633	2.06113	**8.39894**
							4.69399

Bacterial Suspension WITH S-9

	$TiO_2$8	$TiO_2$9	$TiO_2$10	$TiO_2$12	$TiO_2$13	$TiO_2$14	(S-9) PC
Conc 100	1.10219	1.15893	1.11566	1.1013	1.75615	1.27575	**3.38622**
Conc 200	1.34008	1.3729	1.22645	1.37219	2.92085	2.39201	**2.34017**
Conc 400	1.15793	1.3046	1.11214	1.08676	2.46638	1.40647	**1.91376**
Conc 600	0.82746	1.18808	1.0468	1.34458	1.57243	1.30031	**1.39771**
Conc 800	1.21262	1.10052	1.12215	1.15748	1.96345	1.58716	**1.22269**
							1.12751

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4. 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 resistance 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). TiO_2 NPs are inexpensive, biologically and chemically stable, and corrosion-resistive (Xiao *et al*., 2015). Nowadays, the field of materials science consider TiO_2 as an eco-friendly material and promising semiconductor with antimicrobial activity (Gopinath *et al*., 2016 and Periyat *et al*., 2016).

In this study, the antibacterial activity of different concentrations and sizes of the synthesized anatase TiO_2 nanoparticles (NPs) was investigated against MDR strains. Our findings showed that all samples of TiO_2 NPs possessed antibacterial activity against tested strains. Nevertheless, $TiO_2$12 (4.6 nm) and $TiO_2$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). Antibacterial activity of TiO_2 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 inhibition mechanism can be through electrostatic interaction and oxidative stress. Figure.8 summarizes all possible antibacterial mechanisms of TiO_2 NPs against bacterial strains.

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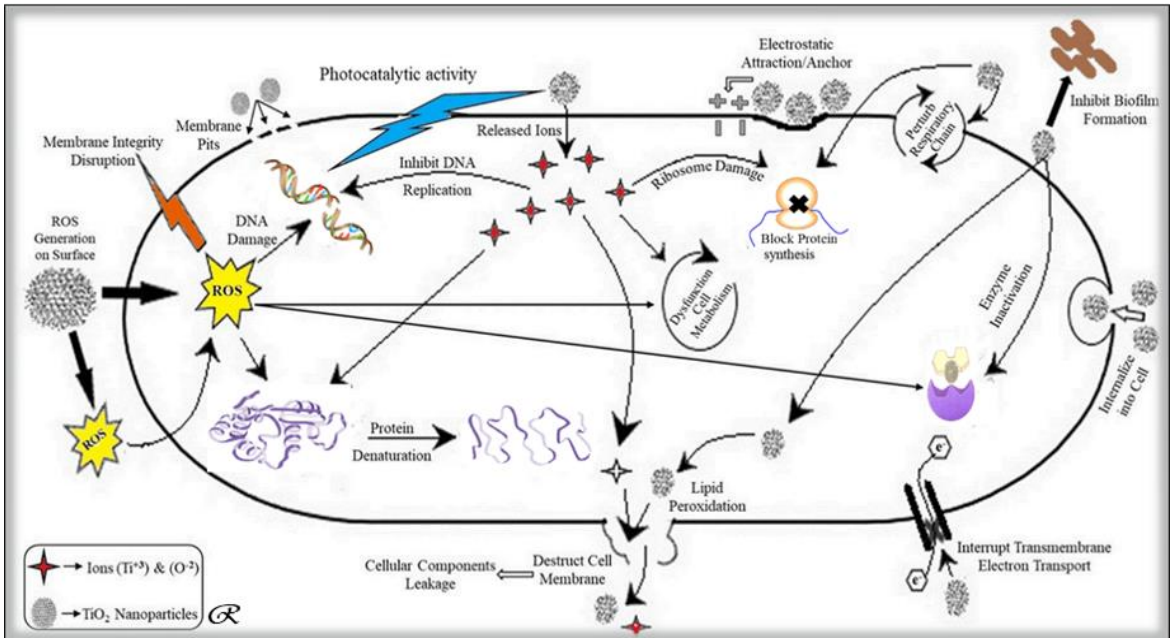

Figure.8. Overview of Possible Antibacterial Activity Mechanisms for TiO_2 NPs

The physiochemical properties of TiO_2 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, and Kumar *et al*., 2017). Many factors 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 had been 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*., 2017). The mixture percentage of de-ionized water (H_2O) to Ethanol (CH_3CH_2OH or C_2H_6O) 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 has 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

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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 and Ramírez-Estrada *et al*., 2016).

Many studies reported that nearly most of the TiO_2 NPs are non-genotoxic/mutagenic (Chen *et al*., 2014b). 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).

5. 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.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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