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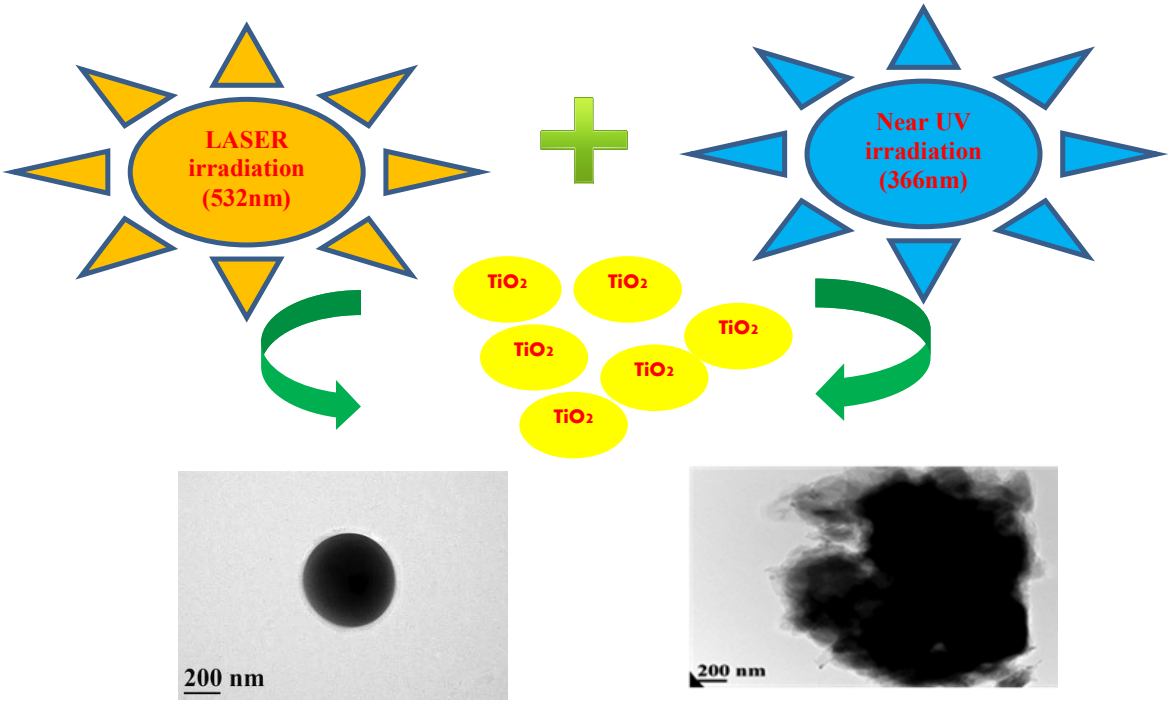

MALDI mass spectrometry for probing the anti- *Staphylococcal* capability of TiO_2 nanoparticles via near-UV and laser irradiation

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Abstract

We report the use of MALDI-MS as an effective tool in investigating the anti-staplylococcal property of TiO_2 nanoparticles under near-UV and for the first time also under Laser irradiation. Since near –UV based photocatalytic activity is toxic to biological cells, it is not feasible for biological studies and hence we propose a more biologically friendly system such as Laser irradiation for inhibition of the clinical pathogen *S. aureus.* Inhouse prepared anatase phase TiO_2 nanoparticles were incubated with the bacterial pathogen and irradiated using near-UV and Laser. The anti-staphylococcal effect was studied using MALDI-MS as an analytical technique in addition to the conventional plate counting method and high resolution TEM. The results showed that the anatase TiO_2 nanoparticles at the concentrations of 0.5µg/mL and 1.5µg/mL showed anti-*Staphylococcal* activity under Laser irradiation (one order reduction in bacterial counts) and Near-UV irradiation (five order inhibition) compared to the control (no TiO_2 NPs) (10^7 cfu/mL). The application of Laser light offers a new prospective for the replacement of near-UV light for sensitive experiments involving biological applications to avoid damage to normal cells. In this study, the ability of TiO_2 nanoparticles (NPs) under Laser and near UV irradiation leading to the anti-Staphylococcal activity was demonstrated using MALDI-MS as the analytical probing tool.

Keywords; TiO_2 nanoparticles, near-UV, laser, MALDI-MS, bacteria,

Introduction

With the emergence of new antimicrobial drugs, the pathogenic bacteria also choose its own way to resist them by molecular changes to overcome the antibacterial agents, this critical situation gives rise nanoparticle (NP) applications. NPs have been promising since the last decade for their antibacterial effect on most pathogenic bacteria which are multi drug resistant. The bactericidal potency of the metallic nanoparticles was achieved due to their small size and high surface to volume ratios leading to higher interaction with bacterial membranes. The combinational effect of the metal nanoparticles with the polymers makes most potent antibacterial agents broad spectrum

catalysts/sensors[1-2]. However, it is gradually being recognized that nanoparticles may have an undesirable impact on the environment and on the ecosystem[3-4]

Bactericidal activity TiO_2/UV reaction and its killing mechanism were also reported by Maness and co-workers and the lipid oxidation reaction was the first evidence proposed as the underlying mechanism for the death of *E. coli*[5]. The anatase titania nanoparticles exhibit higher photo reactivity than that of the rutile[6].The photocatalytic effect of the titanium dioxide was proved in various microorganisms under UV influence, which involves the oxidation of the membrane lipids by reactive oxygen species such as O_2^- and $^{\cdot}OH$ in the presence of O_2 and UV irradiation.

Multi-drug resistant *Staphylococcus aureus* (MRSA) leads to both community and hospital acquired infections which are considered to be a major health problem for human beings[7] . Previous studies have defined several cell-wall constituents of *Staphylococcus aureus* (*S. aureus*), particularly teichoic acid and capsular polysaccharides responsible for the pathogenic properties. None of these antigens except the capsular polysaccharides have provided protective immunity or proven to be reliable diagnostic markers of infection[8].

The use of photocatalysts to destroy organic compounds in contaminated air or water has been extensively reported for the last 25 years. Many organic compounds can be decomposed in aqueous solution in the presence of TiO_2 powders or coatings illuminated under near-UV light or sunlight[9-11]. TiO_2 NPs in the anatase crystal form are known to possess semiconductor properties with a band gap of 3.2 eV or more. The photocatalytic activity results from excitation by light whose wavelength is less than 385 nm, the resulting photon energy generates an electron–hole pair on the TiO_2 surface. Highly oxidative ions such as hydroxyl radicals (OH^-) and superoxide ions (O^-) are produced which are extremely reactive and completely oxidize any organic compound that

they come into contact with[12]. Although the information on the efficacy of TiO_2 NPs as photocatalysts was available long time ago, Matsunaga et al. first reported [13] that microbial cells in water could be killed by contact with TiO_2 NPs upon illumination with near-UV light. Later, the same group successfully constructed a practical photochemical device in which the TiO_2 powder was immobilized onto an acetylcellulose membrane and they reported that an *E. coli* suspension flowing through this device was completely killed[14] . More recently other workers have extended the use of TiO_2 photocatalysis for water treatment applications[15-17].

The main objective of the present study is to synthesize anatase titanium dioxide nanoparticles and study their ability to inhibit the growth of the clinical pathogen- *Staphylococcus aureus* under the influence of Laser and near-UV irradiation. Most of the photocatalytic studies involved only UV irradiation but we also report the use of Laser irradiation in the inactivation studies. Meanwhile, for the first time, we employed MALDI-MS as a feasible tool for studying the anti-staphylococcal activity of TiO_2 NPs, in addition to the standard bacterial counting method and the high resolution TEM studies.

Experimental Methods

Synthesis and preparation of the anatase phase TiO_2 nanoparticles

The TiO_2 NPs were synthesized according to the method described by Qourzal et al. (2008)[18] via the aqueous hydrolysis of titanium (IV) chloride and precipitated using 10% NaOH solution. After 24 h, the hydrated titanium hydroxide deposit was filtered and washed with deionized water until the test for Cl^- ions (silver chloride test, 0.1 M) was negative. The obtained solid was calcined in a furnace (Thermolyne model FB 1315M, Thermo Scientific, Dubuque, IA, USA) for 2 h at 400°C

which is the optimal temperature for the formation of anatase phase. The powder was cooled to room temperature and stored in an amber colored bottle for experiments.

Characterization of the in house synthesized anatase TiO_2 NPs

The TiO_2 nanoparticles after synthesis were characterized using high resolution-TEM (TEM-3010, JEOL, Tokyo, Japan) to obtain information on the particle shape and size. The UV-Vis spectra were also collected to ensure the presence of anatase TiO_2 NPs using an UV-Vis spectrophotometer (U3501, Hitachi, Tokyo, Japan). X-ray diffraction analysis of TiO_2 NPs was also performed to confirm the formation of the TiO_2 NPs.

Bacterial culturing and plate counting methods

Standard *Staphylococcus aureus* (subsp. aureus BCRC 10451) purchased from Bioresource collection and research centre (BCRC), Hsin-Chu, Taiwan was inoculated into the sterile nutrient broth and incubated at 37 °C overnight (Firstek, Orbital Shaking incubator, Firstek Scientific Co, Ltd). The overnight culture was pelleted and washed with sterile distilled water. The final concentration of the cells at 3.2 x 10^7cells/mL in sterile 10 % nutrient broth was used for the experiments. The initial concentration (0h) of cells was estimated by plating 100µL of the bacterial suspension after serial dilution on nutrient agar plates and incubating at 37℃ for 24h and later counting the colonies. Similarly, the bacterial counts in the samples taken at 3h, 6h and 24h intervals after Laser and near-UV irradiation were enumerated by the plate count method. The numbers of bacteria are expressed as total viable counts (TVC) in units of cfu/mL. High resolution-TEM (TEM-3010, JEOL, Tokyo, Japan) was used to image the 6h irradiated bacteria

cells to understand the interaction of the NPs with the bacterial cells. A highly diluted bacterial suspension was spotted on copper grids and dried in vacuum overnight for TEM analysis.

Irradiation methodology

The experimental set up for all the irradiations performed are as follows: the Laser radiation was performed at 532nm from the Class IIIb LASER product which was provided by a portable Laser source. The ultraviolet light source with a wavelength of 366 nm was provided for by two UV GL-58 mineral light lamp purchased from Entela, upland, CA 91786, USA. The entire experiment was performed at room temperature (25-27 ˚C).

The 0g/L (control), 0.5g/L and 1.5g/L concentrations of anatase phase TiO_2 NPs were added to reaction tubes (5mL) containing 3.2 $X10^7$cells/mL of *S.aureus*. One batch was kept under near-UV light irradiation and another set was kept under Laser irradiation. Samples were taken after 3h, 6h and 24h time intervals. The reaction tubes were stirred using small magnetic beads, the entire set-up was mounted on a magnetic stirrer. The tubes were held in a beaker and were irradiated from either side by the respective light near-UV or Laser light source. Samples taken at each time point were analyzed using MALDI-MS and standard plate count method.

Sample analysis using MALDI-TOF MS

Trifluoroacetic acid (TFA) was purchased from Sigma Chemical Co. (St. Louis, MO, USA) and 3, 5-Dimethoxy-4-hydroxycinnamic acid (SA) was purchased from Alpha Aesar (UK). The deionized water purified from a Milli-Q reagent system (Millipore, Milford, MA, USA) was used for all experiments. 500µL of the culture solution was taken for MALDI analysis in Eppendorf tubes and centrifuged at 25,150 x*g* for 5 min. The supernatant was removed and the pellet was

washed in 1mL of distilled water by vortexing for 1 min and then again centrifuged at 15,000 rpm for 5 min. The tubes were vortexed for 2 min and 2.5µL of this sample solution was spotted on a (stainless steel) target plate; each spot was overlaid with 2.5µL of matrix (0.05M sinapinic acid (SA) in 3:1 acetonitrile: water containing 0.1% TFA and air dried. All experiments were performed in triplicates. All mass spectra were obtained using a MALDI-time-of-flight mass spectrometer (Microflex, Daltonics Bruker, Bremen Germany) equipped with a nitrogen laser (337 nm), a 1.25m flight tube and the sample target having the capacity to load 96 samples simultaneously. All spectra were acquired at +20 kV. All mass spectra were acquired in the linear mode with laser energy of 63.2 µJ and 200 laser shots. Figure 1 gives the schematic flowchart showing the methodology applied in the present work.

Results

Inhouse prepared anatase phase TiO_2 nanoparticles were incubated with the bacterial pathogen and irradiated using near-UV and Laser. The TiO_2 nanoparticles were characterized sing HR-TEM, UV and XRD. The anti-staphylococcal effect was studied using MALDI-MS as an analytical technique in addition to the conventional plate counting method and high resolution TEM.

Characterization of TiO_2 NPs

The synthesized (anatase) TiO_2 NPs were characterized by the high resolution TEM (Fig. 2(a)) which indicates that the particle sizes are in the ranges of 10-20 nm (the inset Figure in Fig. 2(a)). Their symmetries were dual being either globular or oblong. The UV spectrum of the TiO_2 NPs are shown in Fig. 2(b) showing an absorbance peak at **387nm** which confirmed the successful synthesis of TiO_2 NPs[19]. Fig. 2(c) displays the XRD patterns of the TiO_2 NPs exhibited strong

diffraction peaks at 25° and 48° indicating TiO_2 NPs in the anatase phase. All peaks are in good agreement with the standard spectrum (JCPDS no.: 84-1286) for the anatase TiO_2 NPs[20].

Evaluating the anti-Staphylococcal effect by using MALDI-TOF MS

Staphylococcus aureus cells were incubated at 0g/L (control, absence of TiO_2 NPs), 0.5g/L and 1.5g/L concentrations of anatase TiO_2 NPs under Laser and near-UV irradiation. The impact of the TiO_2 NPs when irradiated by two different light sources was assessed after 3h, 6h and 24h of exposure. Samples were analyzed using MALDI-MS, the results showed that after 3h irradiation under laser light compared to the control (0g/L, no TiO_2 NPs) (Fig. S1A(a), supporting information), no significant reduction or anti-Staphylococcal effect was observed at 0.5g/L concentration (Fig. S1A(b), supporting information) and 1.5g/L concentrations (Fig. S1A(c), supporting information) of TiO_2 NPs. Rather, it was observed that the addition of the TiO_2 NPs resulted in the appearance of some protein peaks at *m/z* values of 3659.6, 7220.2, 9801.2, 10172.3 and 12058.8. But, one predominant bacterial peak at *m/z* 6232 was observed to be significantly quenched at 0.5g/L and 1.5g/L NPs concentration. In the case of bacteria irradiated in the presence of near-UV light at 0 g/L (control, no NPs), 0.5g/L and 1.5g/L concentrations of anatase TiO_2 NPs, the samples analyzed at 3h showed no significant changes in the MALDI-MS spectra (Fig. S1B(a-c), supporting information). At 0.5g/L concentration (Fig.S1B(b)) it was observed that few additional protein peaks appeared at *m/z* 7219, 7365 and 8115. However, under Laser irradiation it was observed that 1.5g/L concentration (Fig. S1A(c)) also led to the disappearance of the peak at m/z 6224 similar to the trend observed in the case of near-UV light illumination.

Even, after 6h of exposure under laser light illumination it was observed that the laser light did not show any bactericidal effect in the absence of the NPs (Fig. S2A(a)). Meanwhile, 0.5g/L

concentrations were observed to show enhancement in the bacterial peaks (Fig. S2A(b)) with the only exception being the m/z 6224 peaks which showed significant quenching. Even at 1.5g/L concentrations only a similar pattern (no decrease) was observed (Fig. S2A(c)). Similarly, it was observed that under near-UV light irradiation the control (Fig.S2B(a)) even after 6h of exposure in the absence of the NPs showed no bactericidal activity. 0.5 g/L concentration (Fig. S2B(b)) of TiO_2 NPs did not show any bactericidal activity either, instead more bacterial peaks were observed, but at 1.5g/L TiO_2 NPs concentration the 6000 Da peak clusters showed significant reduction and the other peaks also showed marked reduction in peak intensity (Fig. S2B(c)).

Fig.3A gives the MALDI-MS spectra of the 24h irradiated bacterial sample, as we can seen from Fig. 3A(a) still the bacterial cells in the absence of the NPs show no bactericidal activity. This proves that Laser light in the wavelength of 532nm is not harmful to bacterial cells and the anti-Staplylococcal or pathogenicidal effect observed was solely due to the TiO_2 NPs. Gradual reduction in the bacterial signals and peak intensities was observed at 24h in *S.aureus* cells incubated with 0.5g/L TiO_2 NPs (Fig.3A(b)) and 1.5g/L TiO_2 NPs (Fig.3A(c)) under Laser illumination. And under near-UV light illumination an even more drastic reduction of bacterial signals was observed both at 0.5g/L TiO_2 NPs (Fig. 3B(b)) and 1.5g/L TiO_2 NPs (Fig. 3B(c)) concentrations. Fig.4A gives the comparative spectra showing the effect of Laser and UV-light on the bacterial cells in the absence of the TiO_2 NPs. As can be observed from Fig. 4A(A) and Fig. 4A(b) neither Laser nor UV Light showed any pathogenicidal activity. But, as Fig. 4A(c) shows, Laser light irradiation in the presence of 1.5g/L of TiO_2 NPs did show some reduction especially at the 6KDa peak cluster. And near UV-light illumination combined with 1.5g/L of TiO_2 NPs (Fig. 4A(d)) showed remarkable reduction showing complete inhibition of all bacterial peaks. Thus the MALDI-MS studies prove the anti-staphylococcal ability of TiO_2 NPs under near UV light

illumination and Laser light irradiation, as revealed by the significant changes in the spectra, shown by disappearance of prominent bacterial protein peaks following treatment.

Evaluating the anti-Staphylococcal effect using high resolution-TEM

The high resolution-TEM studies showed that the control cells placed under near-UV and Laser light illumination showed no damage as can be observed in Fig. 4B(a). In the case of the Laser irradiated cells in the presence of 1.5g/L of TiO_2 NPs it was observed that the cells appeared less dense than the control cells and we can see the TiO_2 NPs surrounding the bacterial cells and interacting with them. We know from the MALDI-MS analysis that 1.5g/L concentrations were pathogenicidal and showed reduction in bacterial signals under Laser irradiation. Fig. 4B(c) gives the high resolution-TEM micrograph showing the damaged, ruptured bacterial cells irradiated under near-UV light illumination when incubated with 1.5g/L of TiO_2 NPs. As can be observed the cell contours could not be seen distinctly and the cells appeared lysed and totally damaged. These microscopic observations further prove the trend observed in the MALDI-MS analysis that the TiO_2 NPs concentrations were highly photocatalytic under near UV light illumination and the trend extended under Laser light leading to anti-Staphylococcal activity.

Evaluating the anti-Staphylococcal effect using standard plate counting method

The samples obtained after incubating the bacteria with two different concentrations of TiO_2 NPs showed that under Laser irradiation, no pathogenicidal effect was observed at 3h at both 0.5g/L and 1.5g/L TiO_2 NP concentrations. However, it was observed that beyond 6h marginal reduction in counts compared to the control (0g/L) set (Fig. 5A) was noticed and at 24h a one order reduction in bacterial counts was observed in the case of both 0.5g/L and 1.5g/L treated bacterial cells. Under Laser irradiation it was observed that both 0.5g/L and 1.5g/L TiO_2 NPs concentrations

showed almost the same effect. This matches with the MALDI-MS which also indicated reduction in bacterial peaks only beyond 6h and maximum at 24h. In addition as correctly reflected by the MALDI-MS results, mere laser light irradiation (without TiO_2 NPs) did not show any inhibitory effect which can be seen in the increase in cell number in the control.

In the case of the near-UV light irradiated samples (Fig. 5B), it was observed that rapid reduction in bacterial numbers started even as early as at 1h and 3h, a reduction nearing one order compared to the control was observed and at 6h, when the control counts were 10^7 cfu/mL the 0.5g/L and 1.5g/L treated cells showed counts equivalent to 10^8 cfu/mL. Also, up to 6h it was observed that both NPs concentrations behaved the same exhibiting similar pathogenicidal effect. However, beyond 6h and at 24h it was observed that 1.5g/L concentrations showed maximum inhibitory property (10^2) compared to 0.5g/L concentrations which also showed high pathogen inhibition (10^3) compared to the untreated control cells. Unlike the laser light, near-UV light was observed non-supportive to cell count increase with incubation time. But, no reduction was seen in this case either in the absence of the NPs. This was an interesting observation noticed in the pattern of growth of control cells under Laser and near-UV light illumination.

The results showed that based on the MALDI-MS and standard plate counting methods, that the pathogenicidal/anti-Staphylococcal effect was exhibited by 0.5 g/L TiO_2 NPs and 1.5 g/L TiO2 NPs under Laser irradiation under longer incubation more than 6h. At 24h a one order of magnitude difference was observed. And in the case of near UV light illumination a steady decrease in bacterial number was observed even as early as <3h and at 24h almost five orders of magnitude reduction was observed in cells incubated with 1.5g/L of TiO_2 NPs and four orders of magnitude reduction was observed in the cells incubates with 0.5g/L of TiO_2 NPs.

Discussion

Certain materials (oxides, sulphides, etc.) act as photoconductors on illumination with near-UV photons. If the two photoinduced charge carriers (electrons and holes) do not recombine, they can reach the surface and react with chemisorbed species giving reduction/oxidation reactions[21-22]. The naturally abundant titanium oxide (TiO_2) exists in three crystalline forms: brookite (orthorhombic), anatase (tetragonal) and rutile (tetragonal). Anatase has a higher degree of tetragonality than the rutile structure and it is also less closely packed[23-24]. In recent years, TiO_2, especially the anatase phase (band gap, 3.2 eV), is well known as a semiconductor with strong photocatalytic activity and has a great potential for applications such as environmental purification, decomposition of carbonic acid gas and generation of hydrogen gas[25]. This property of TiO_2 has been applied in the removal of organic chemicals in effluents[26]. Photocatalytic oxidation has been developed as an environmentally benign approach to waste-water remediation using natural sunlight. Commercial processes for waste-water treatment using TiO_2 based photocatalytic oxidation are now available[27]. Gopal et al. report the successful use of anatase type of TiO_2 for the inactivation of bacteria using the anatase TiO_2 thin films[28-31].

The mechanism of photocatalytic inhibition is that upon excitation by light whose wavelength is less than 385 nm, the photon energy generates an electron–hole pair on the TiO_2 surface. The hole in the valance band can react with H_2O or hydroxide ions adsorbed on the surface to produce hydroxyl radicals (OH^-), and the electron in the conduction band can reduce O_2 to produce superoxide ions radicals (O^-). Both holes and OH^- are extremely reactive with contacting organic compounds and are able to completely oxidize these compounds[12]. Also these reactive oxygen species (ROS) such as hydroxyl radical, superoxide anions and hydrogen peroxide

generated on the surface have been reported to be responsible for the inactivation of the microorganisms[32]. The ROS (not only OH radical but hydrogen peroxide (H_2O_2), hydro peroxyl radical (HO_2) and singlet oxygen (O_2)[33-35] targets the outer most membrane of the bacterial cells and disrupts the membrane proteins leading to lipid peroxidation. Gopal et al. (2007) report that two mechanisms seem to be operating, one through the direct oxidation by the positive holes and the other indirect oxidation by the formation of free radicals[36]. Direct oxidation by the positive holes results in oxidative stress within the cells which induces the formation of free radicals, which can bring about cell death[37]. Indirect oxidation involves the production of free radicals by the electron holes on the oxide surface. Although it is not known which of these mechanisms is operating, the ultimate cell inactivation or inhibitory effect seems to be either through the production of free radicals by the electron holes or by the cells themselves in response to the stress conditions or a combination of both .

So far, only UV and near UV light irradiation have been reported to excite the TiO_2 NPs to exhibit photocatalytic activity, leading to killing of bacteria[38]. In the current study although we have no evidence that the Laser irradiation increased the photocatalytic activity of TiO_2, we do have evidences that it was able to result in bringing about inactivation of the bacterial pathogen. Although the photocatalytic effect in the presence of near UV is significantly higher than the anti-staphylococcal activity observed under Laser irradiation, this study points out to the possibility of hiring the services of Laser belonging to the class of biologically safe laser light as the one used in this experiment for sensitive experiment involving biological cells, since near UV light is not entirely trust worthy. Our experiments prove that the near-UV light did not show any bactericidal effect but did show a bacteriostatic effect where we saw that the growth curve became stationary. However, the Laser light used in this study showed no effect on bacterial cells and was totally

harmless to biological cells. This work paves the way for use of biologically safe Lasers for experiments involving biological trials and specimens. Visible light photocatalysis has been reported[39-43] but no reports confirming the role of lasers in promoting photocatalytic activity is reported so far. The laser is reported to be able to activate drugs leading to their higher activity leading to bacterial killing[44]. We believe that the laser irradiation played am indirect role in activation of the TiO2 NPs to exhibit more killing effect. Also, it is possible that the killing could be through the absorbtion of heat from the lasers by the NPs and their transfer to the bacterial cells, leading to death. More investigations in this direction will help in throwing light into this phenomenon.

CONCLUSION

The present study demonstrated the anti-Staphylococcal activity of the synthesized anatase TiO_2 nanoparticles probed by the MALDI-MS, high resolution TEM and standard plate counting method under Laser and near UV irradiation. The results obtained from MALDI-MS and standard plate counting methods indicating that 0.5 g/L and 1.5 g/L of TiO_2 nanoparticles exhibited anti-Staphylococcal effect via Laser irradiation for longer incubation/reaction time (more than 6h). At 24h, one order of magnitude difference was observed. In the case of near UV light illumination a steady decrease in bacterial number was observed even as early as <3h and at 24h almost five orders of magnitude reduction was observed in cells incubated with 1.5g/L of TiO_2 nanoparticles and four orders of magnitude reduction was observed in the cells incubated with 0.5g/L of TiO_2 nanoparticles.

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Figure captions

Fig. 1. Schematic diagram showing the experimental methodology.

Fig. 2.(a) High resolution-TEM images of TiO_2 nanoparticles with the inset Figure showing the size distribution of particles to be in the range of 10-20 nm. (b) UV-Vis Spectrum of the anatase TiO_2 NPs showing the maximum absorption at 290 nm. (c) X-ray diffraction (XRD) graph of TiO_2 NPs confirming the presence of anatase at 2θ angles of 25^o and 48^o.

Fig. 3A MALDI-MS spectra showing the effect of different concentrations (a) 0 g/L (control) (b) 0.5g/L (c) 1.5g/L of anatase phase TiO_2 NPs on *S.aureus* after 24h exposure to Laser illumination.

Fig. 3B MALDI-MS spectra showing the effect of different concentrations (a) 0 g/L (control) (b) 0.5g/L (c) 1.5g/L of anatase phase TiO_2 NPs on *S.aureus* after 24h exposure to near UV illumination.

Fig. 4A. MALDI - MS spectra of *S.aureus* in presence of (a) Laser irradiation (6h) with 0g/L of TiO_2 NPs (b) near-UV irradiation(6 h) with 0 g/L of TiO_2 NPs (c) Laser irradiation(6h) with 1.5 g/L of TiO_2 NPs (d) near-UV irradiation (6h) with 1.5 g/L of TiO_2 NPs.

Fig. 4B(a) TEM image of *S.aureus* grown in the absence of TiO_2 NPs (control) showing normal micrograph of cells. (b) TEM image of *S.aureus* incubated with 1.5g/L of TiO_2 NPs for 24h under Laser irradiation showing aggregation of TiO_2 NPs around the bacterial cells and also cells were appearing less dense compared to the control cells. (c) TEM image of *S.aureus* bacteria incubated with 1.5g/L of TiO_2 NPs for 24h under near near-UV irradiation showing complete lysis of cells resulting in cell debris; note that no TiO_2 NPs were observed surrounding the debris; it indicates that the TiO_2 NPs had penetrated the cells.

Fig. 5A Graph showing total bacterial counts of *S.aureus* incubated with 1.5g/L concentration of anatase TiO_2 NPs at different time intervals under Laser illumination.

Fig. 5B Graph showing total bacterial counts of *S.aureus* incubated with 1.5g/L concentration of anatase TiO_2 NPs at different time intervals under near-UV illumination.

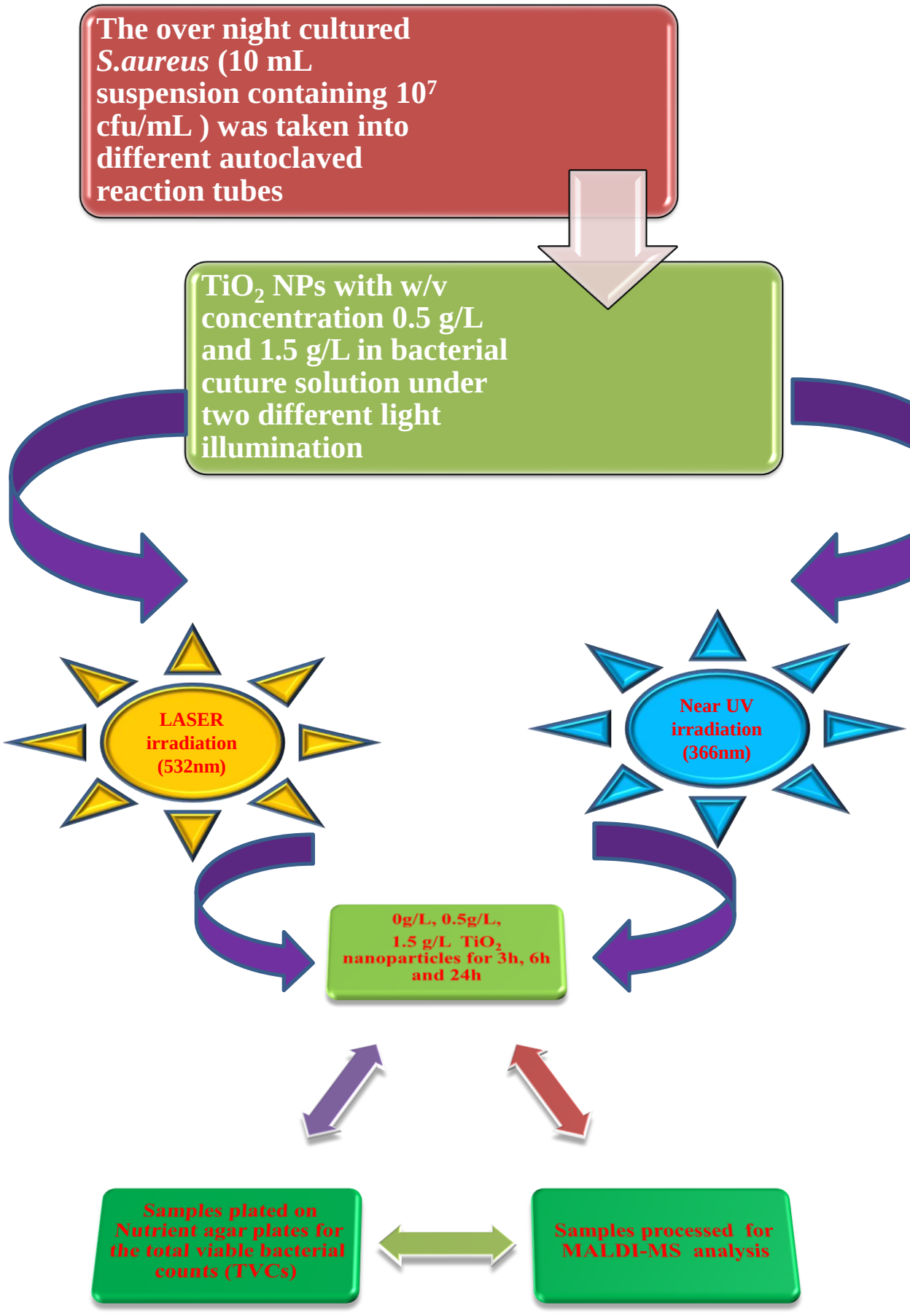
Supporting Figure Captions

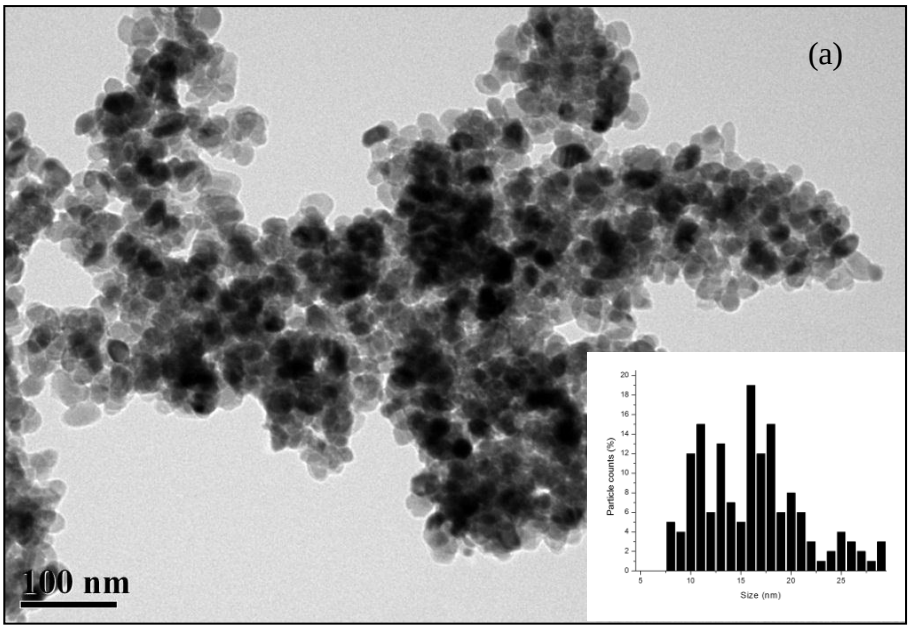
Fig. S1A. MALDI-MS spectra showing the effect of different concentrations (a) 0 g/L (control) (b) 0.5g/L (c) 1.5g/L of anatase phase TiO_2 NPs on *S.aureus* after 3h exposure to Laser illumination. B. MALDI-MS spectra showing the effect of different concentrations (a) 0 g/L (control) (b) 0.5g/L (c) 1.5g/L of anatase phase TiO_2 NPs on *S.aureus* after 3h exposure to near UV illumination.

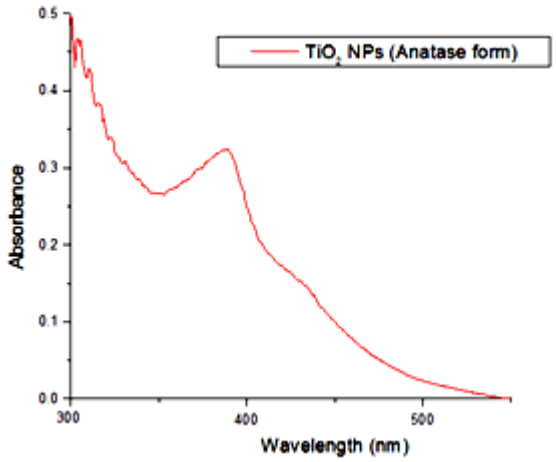
Fig. S2A. MALDI-MS spectra showing the effect of different concentrations (a) 0 g/L (control) (b) 0.5g/L (c) 1.5g/L of anatase phase TiO_2 NPs on *S.aureus* after 6h exposure to

Laser illumination. B. MALDI-MS spectra showing the effect of different concentrations (a) 0 g/L (control) (b) 0.5g/L (c) 1.5g/L of anatase phase TiO_2 NPs on *S.aureus* after 6h exposure to near UV illumination.

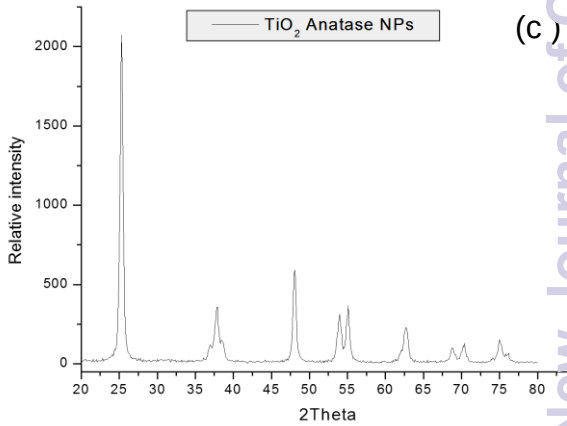
Fig 1







(b)



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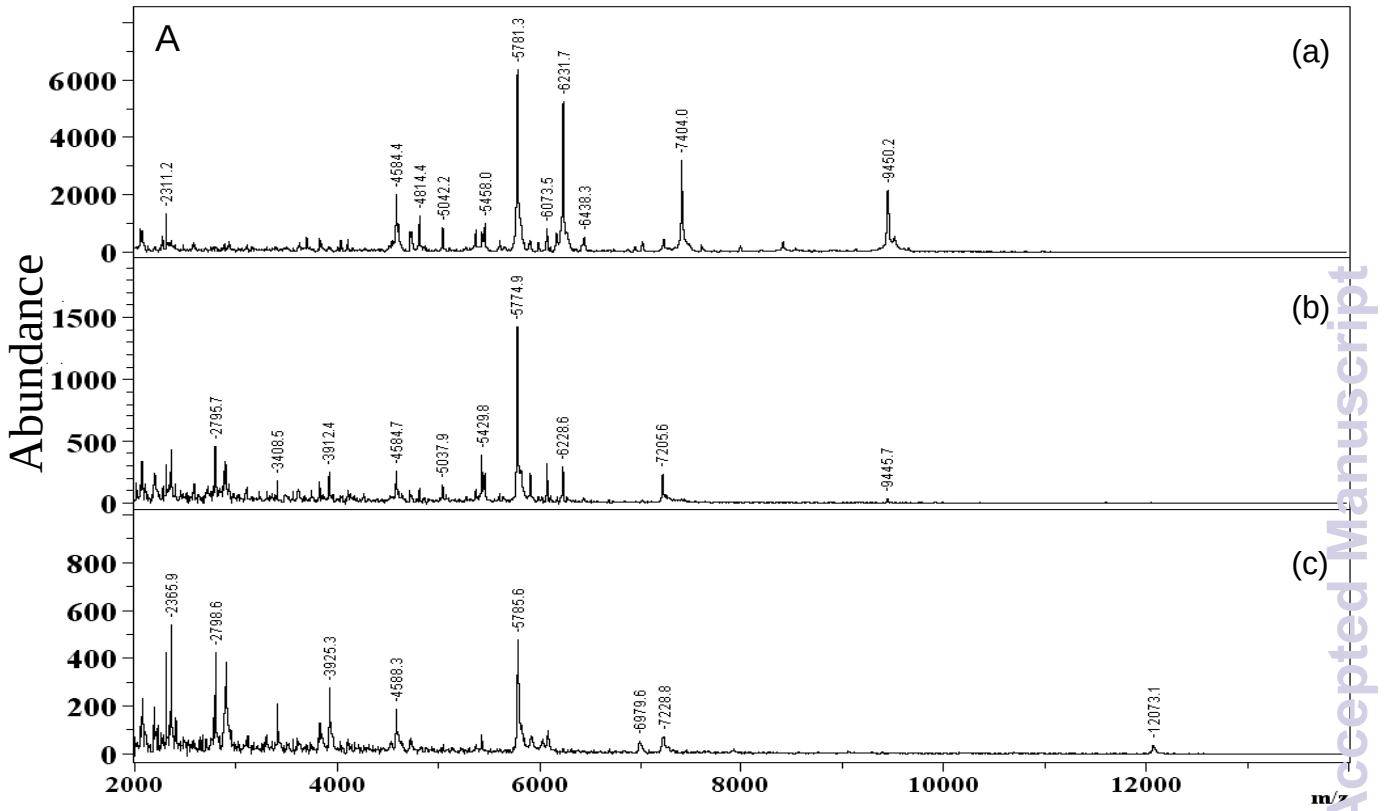

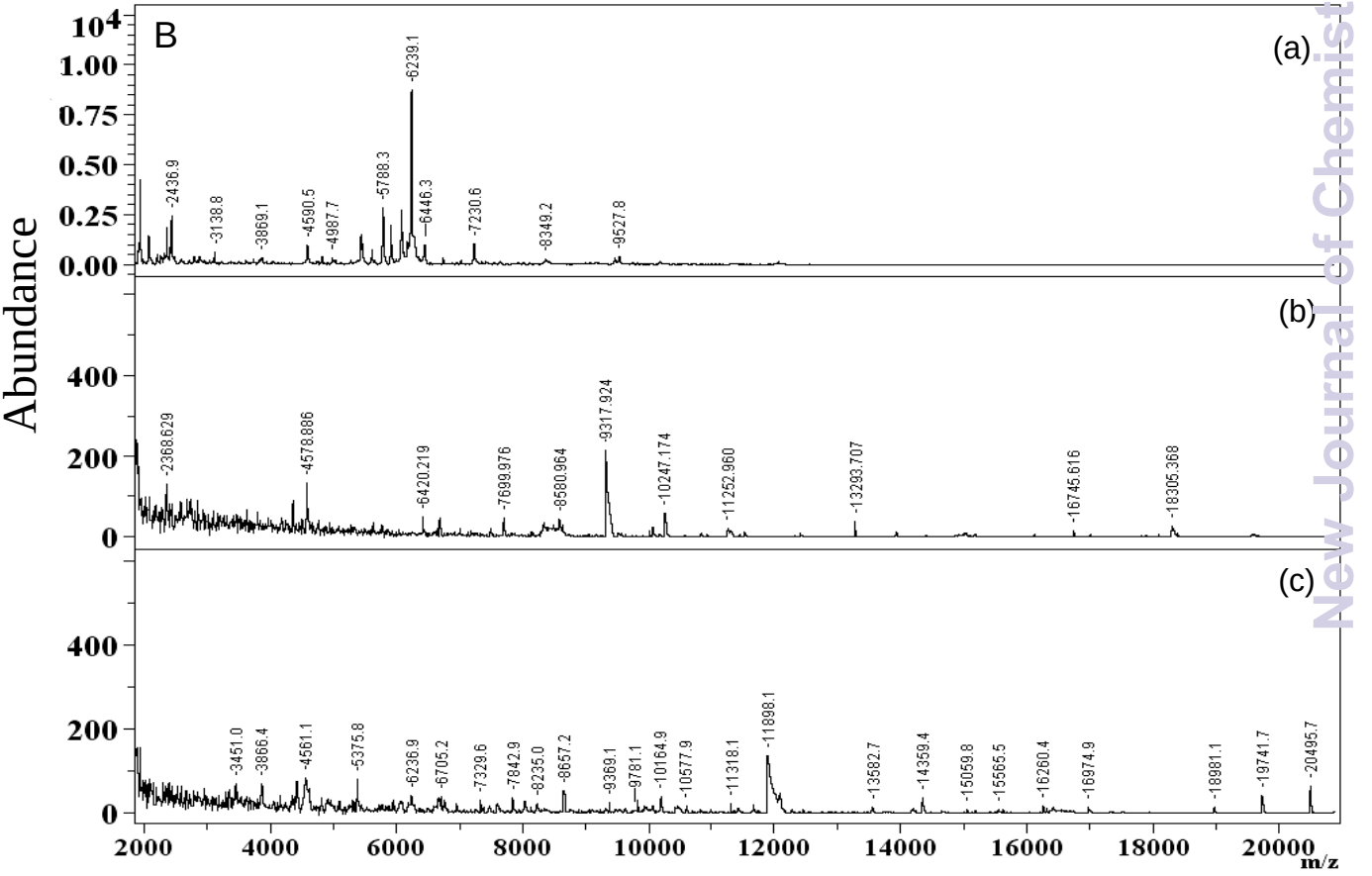

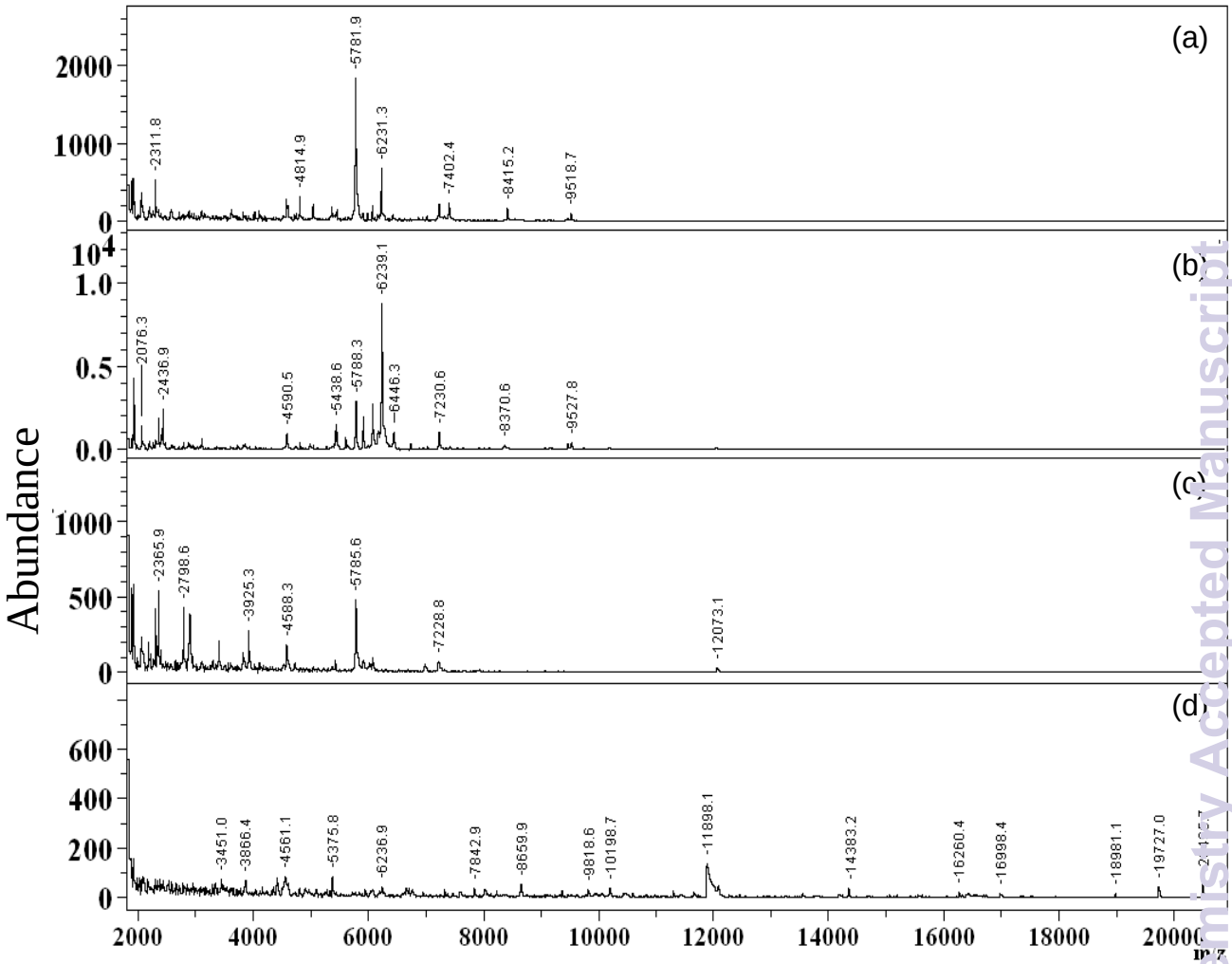

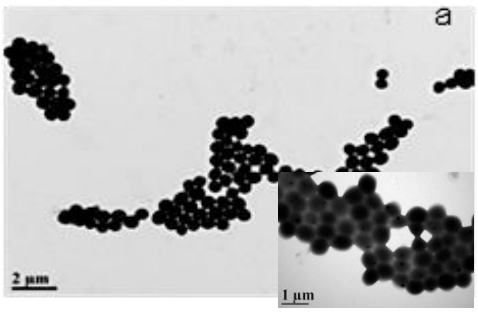

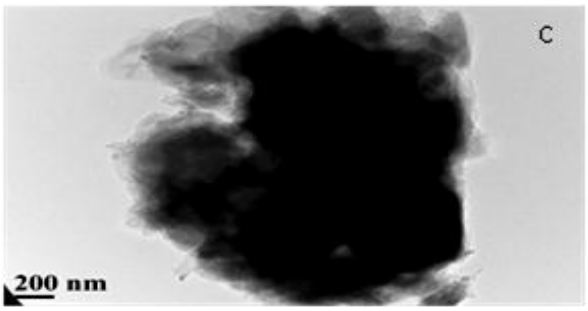

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MALDI mass spectrometry for probing the anti- *Staphylococcal* capability of TiO_2 nanoparticles via near-UV and laser irradiation

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Fig 5A

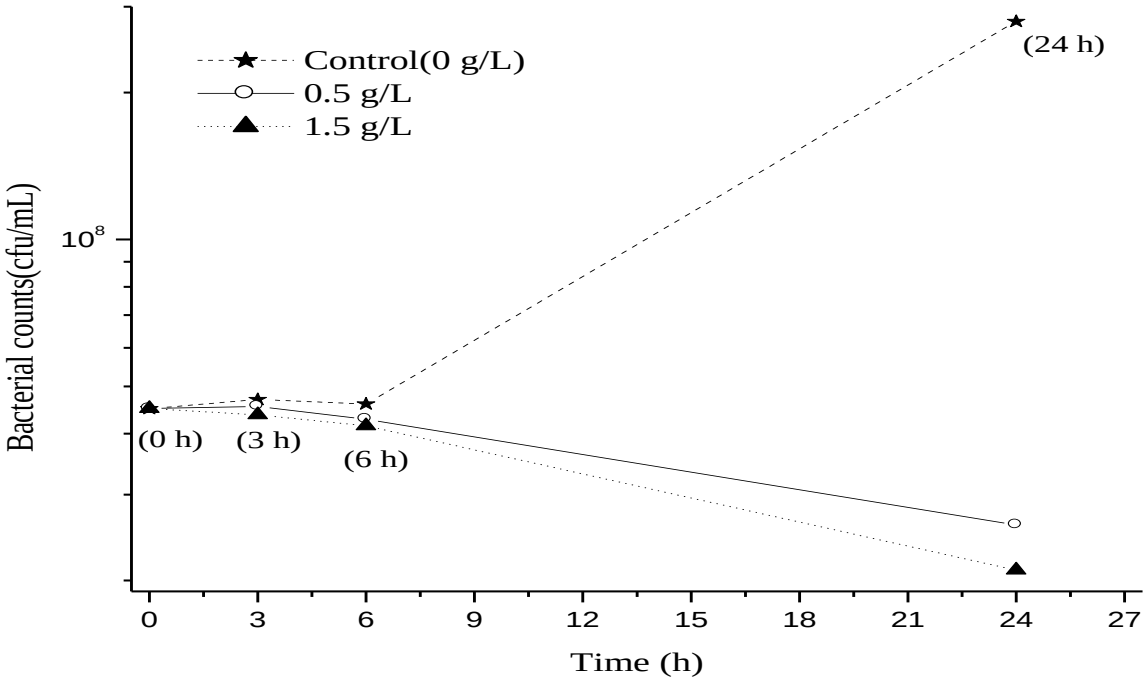

Fig 5B

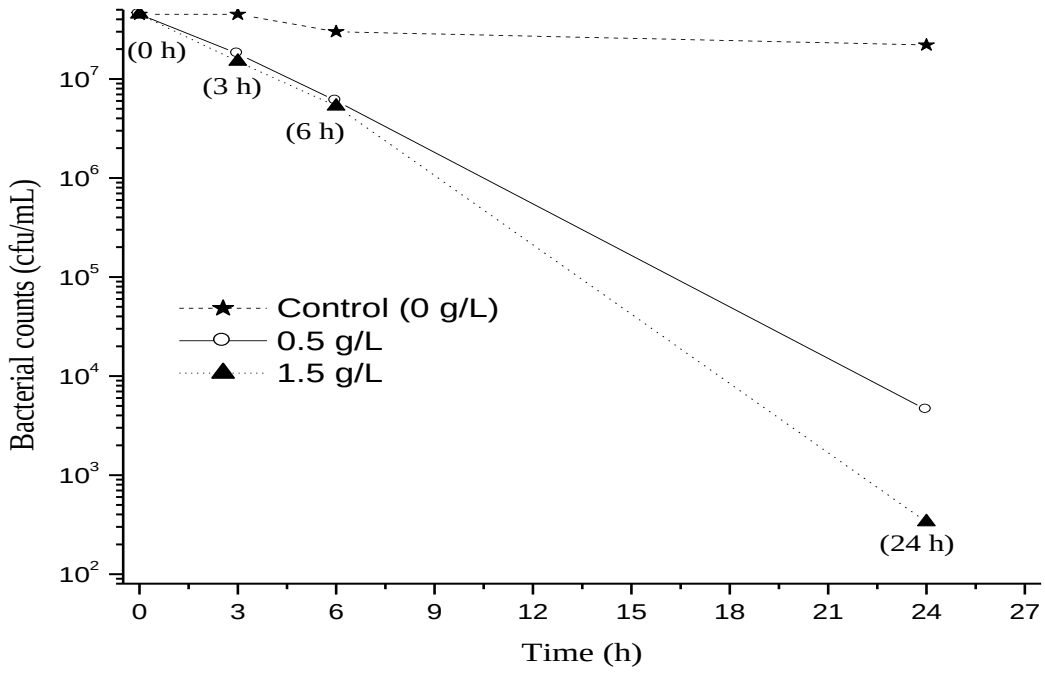

Fig S1 A

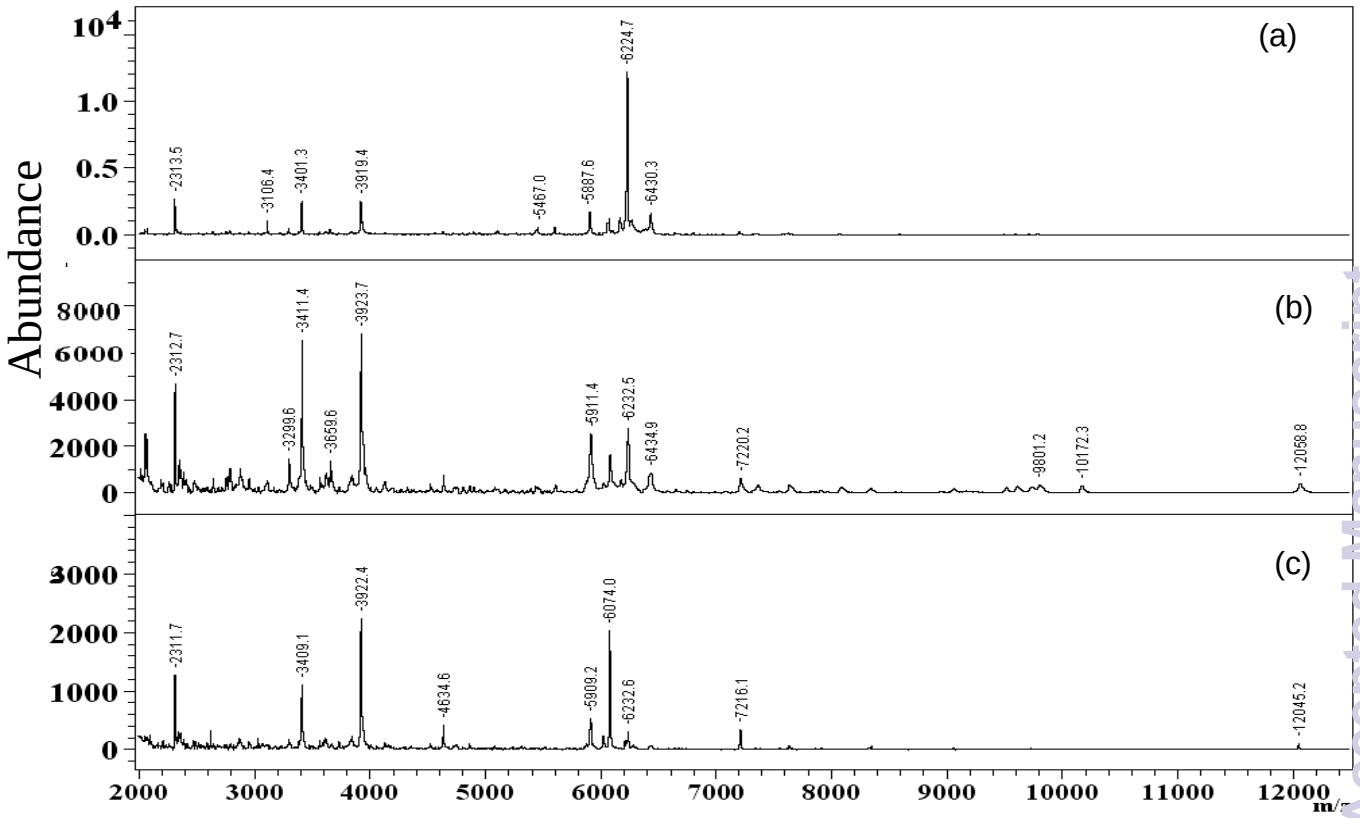

Fig S1 B

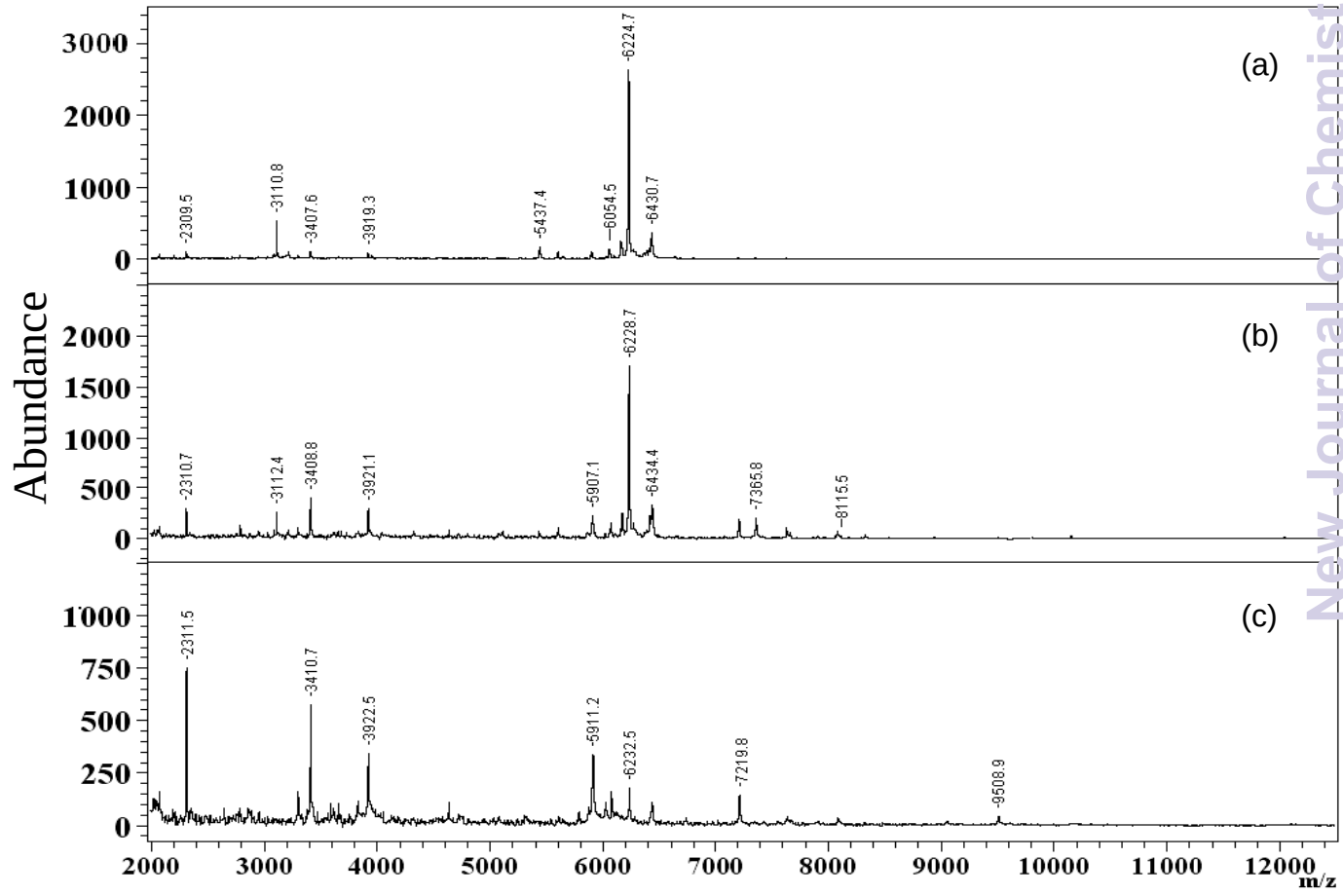

Fig S2A

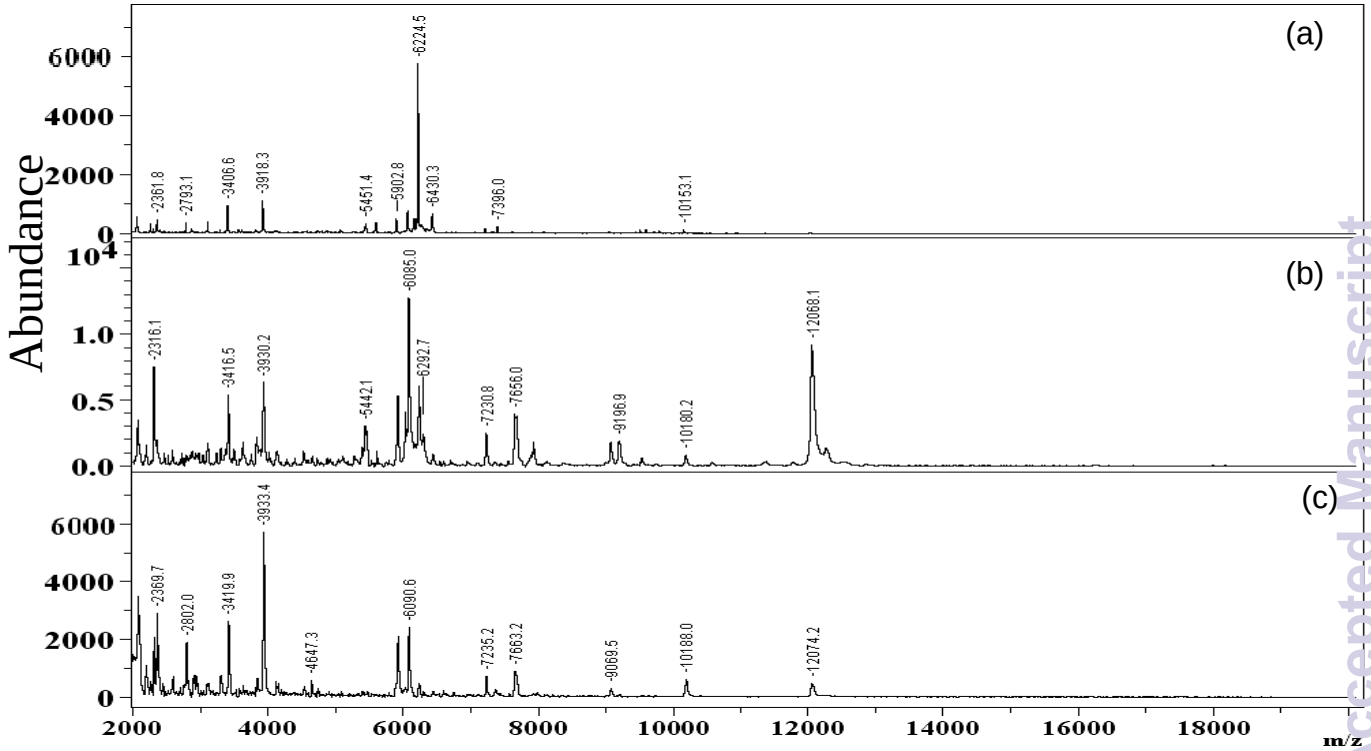

Fig S2B