Characterization and bacterial toxicity of lanthanum oxide bulk and nanoparticles

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Abstract: This study evaluated the bacterial toxicity of lanthanum oxide micron and nano sized particles using shake flask method against gram-positive (Staphylococcus aureus) and gram-negative (Escherichia coli, Pseudomonas aeruginosa) bacteria. Particle size, morphology and chemical composition were determined using scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS). Results indicated that lanthanum oxide nanoparticles showed antimicrobial activity against Staphylococcus aureus, but not against Escherichia coli and Pseudomonas aeruginosa. It was speculated that lanthanum oxide produced this effect by interacting with the gram-positive bacterial cell wall. Furthermore, lanthanum oxide bulk particles were found to enhance the pyocyanin pigment production in Pseudomonas aeruginosa.

Keywords: lanthanum oxide: nanoparticles: bacterial toxicity: pyocyanin; rare earths

In recent years, the rapid development in the field of nanotechnology has provided a wide range of application in fields of material, medical, computer, bio-sciences and information technology. Formulation of nanomaterial based antimicrobial agents has received much attention due to its unique and distinctive physical, chemical and biological properties due to its size effect and large surface to volume ratio. A number of studies have been focused on the preparation of antimicrobial agents using nanomaterials and nanocomposites[1–5]. Interestingly, inorganic based antimicrobial agent has shown better stability under higher temperature and pressure than organic based antimicrobial agents. Over the past few years, numerous studies have been undertaken to investigate the antimicrobial activity of metal and metal oxide nanoparticles[6–8]. Among these, a number of reports on the antimicrobial activities of silver and zinc oxide nanoparticles have been reported[9–12]. New nanoparticles are being investigated for their antimicrobial properties and to develop alternative antimicrobial agents to control bacterial infections.

Rare earth elements, characterized by their high density, high melting point, high thermal conductance and conductivity, possess unique physical and chemical properties due to their 4f orbital electron and this has been extensively applied in electronics, medical, biomedial and agronomic fields[13,14]. Lanthanum oxide (La₂O₃) is a rare earth metal oxide, which has a band gap of 4.3 eV and the lowest lattice energy with high electric constant[15]. Also, it has been in use as a p-type semiconductor and has several other applications in areas of electronics, fuel cells, optics, magnetic data storage, ceramics, catalysis, automobiles, biosensor, water treatment and biomedicine[16–18]. The possible applications of these materials has not been fully explored especially in the field of biomedical sciences. To the best of our knowledge, no studies have looked into the bacterial toxicity of lanthanum oxide bulk (La₂O₃ bulk) and lanthanum oxide nanoparticle (La₂O₃ NP).

In this report, we have characterized the size, structure and chemical composition of La₂O₃ bulk and La₂O₃ NP’s using scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS) techniques. Furthermore, these materials were analyzed for their bactericidal property against some potential pathogenic clinical isolates like S. aureus, E. coli and P. aeruginosa.

1 Experimental

1.1 Materials
La₂O₃ bulk (Ottokemi, Mumbai, India), La₂O₃ NP (Nanoshel, Panchkula, India), nutrient broth (Himedia, Mumbai, India), nutrient agar (Himedia, Mumbai, India), nutrient agar (Himedia, Mumbai, India), chloroform (Rankem, Faridabad, India) and hydrochloric acid (Merck, Mumbai, India).

1.2 Characterization
The surface topography of the sample was analyzed by scanning electron microscopy (SEM). The samples were spread on the carbon tap and analyzed with an accelerating voltage of 20 kV using Quanta 200 FEG SEM. The chemical...
composition of the sample was obtained by energy dispersive X-ray spectroscopy analysis (EDS).

1.3 Bacterial toxicity assessment assay

Toxicity of La$_2$O$_3$ (bulk and NP) against *S. aureus*, *E. coli* and *P. aeruginosa* bacteria was investigated using shake flask method.

La$_2$O$_3$ bulk and nanoparticles at a final concentration of 10 mg/ml were added to separate culture flasks containing the pathogens *S. aureus*, *E. coli* and *P. aeruginosa*. The flasks were then incubated at 37 °C for 24 h in a shaker water bath set at 100 r/min. Prior to incubation and at the end of 24 h incubation, 1 ml samples from each culture flasks were taken, serially diluted twice, and 100 µL of this diluted sample was spread on nutrient agar plates. These plates were incubated at 37 °C, to obtain 0 and 24 h colony counts. Untreated controls were also included in this study.

Colony counts at 0 and 24 h were used to determine the effect of the test items (La$_2$O$_3$ bulk or NP) on bacterial viability.

1.4 Quantitative evaluation of pyocyanin production

Preliminary experiments showed that La$_2$O$_3$ had some effect on the pyocyanin production by *Pseudomonas*. This was further investigated by evaluating the effects of La$_2$O$_3$ bulk and NP on pyocyanin production. Pyocyanin pigment was extracted in 6 ml of chloroform and 2 ml of 0.2 mol/L hydrochloric acid before and 24 h post treatment. The optical density of the extract was measured at 520 nm. The absorbance value of each sample was multiplied by 17.072 to express the concentration of pyocyanin production in micrograms per milliliter of culture supernatant[19–21].

Three independent experiments were performed to check the reproducibility of the results.

2 Results and discussion

2.1 SEM and EDS analysis

The size and surface morphology of both La$_2$O$_3$ bulk and NPs were examined using SEM. The SEM image of La$_2$O$_3$ bulk and NPs are given in Figs. 1(a) and (b). The sizes of La$_2$O$_3$ bulk particles were in the region of 1 µm as seen in Fig. 1(a). From Fig. 1(b) it is clear that these nanoparticles are almost spherical in shape and are uniform in size of about 100 nm. The chemical composition of the La$_2$O$_3$ bulk and NP samples were analyzed by energy dispersive spectrum (EDS) and are shown in Figs. 1(c) and (d), respectively. The peaks reveal the presence of lanthanum (La) and oxygen (O).

2.2 Bacterial toxicity assays

2.2.1 Toxicity to *S. aureus* La$_2$O$_3$ NPs showed significant toxicity against *S. aureus*. There were $2.6 \times 10^8$ cfu
before and only 1.8×10⁷ cfu following treatment with La₂O₃ NPs. This activity was not observed with La₂O₃ bulk material (see Table 1). The specific antibacterial activity of La₂O₃ NP against *S. aureus* may be attributed to lanthanide ions suppressing the activities of Ca²⁺ ions. Isomorphic capabilities of lanthanide ions replace the Ca²⁺ in the binding sites of staphylococcal nucleases and interrupt the activation and prevent the growth metabolism of the *S. aureus*. If the above mechanism holds good, the toxicity against *S. aureus* should be observed following treatment with both bulk material as well. But our results clearly suggest that La₂O₃ bulk did not have toxicity against *S. aureus*. Therefore, some other mechanisms, other than calcium ion replacements may be involved in the bactericidal activity against *S. aureus*. Another possible explanation for of bacterial toxicity is via the induction of free radicals. Generations of free radicals like super oxide and hydroxyl ions mainly affect the macromolecules (DNA, lipids and proteins). Generally lanthanide oxides are known for their strong production of OH radicals. The generation of free radical in rare earth elements are ranked in the order of La₂O₃>Nd₂O₃>Sm₂O₃>Yb₂O₃>>CeO₂[23]. Furthermore, another study reported the reactive oxygen species produced by the lanthanum, damaged the hepatic nuclei and mitochondrial[13]. In this study, the bactericidal properties against *S. aureus* may not be fully attributable to the generation of free radicals, because other bacteria tested were not affected.

Furthermore, it is also possible that bactericidal effect against *S. aureus* induced by La₂O₃ NP may be due to the interaction between the positively charged NP and negatively charged cell wall[24–28]. It therefore appears that the activity of lanthanum NP against *S. aureus* is multi-factorial and further research is necessary.

A brief postulated mechanism is depicted in Fig. 2, which hypothesize that the bacterial toxicity may be due to the electrostatic interaction between the positively charged NP and negatively charged *S. aureus*. Overall charge of the *S. aureus* is negative due to the presence of excessive carboxylic acid in the cell wall. Therefore, the positively charged NPs are easily attracted towards the bacteria and are attached to the bacterial cells. Attached NPs mechanically damaged the bacterial cell wall and penetrated into the cell. Once the cell wall is damaged all cell constituents leak and cause cell death.

### Table 1 Quantitative evaluations of *S. aureus* bacteria growth at 0 and 24 h of treatment with La₂O₃ bulk, La₂O₃ NP and control (cfu/ml)

<table>
<thead>
<tr>
<th>Experiment</th>
<th>0 h untreated control</th>
<th>24 h untreated control</th>
<th>0 h treatment with La₂O₃ bulk</th>
<th>24 h treatment with La₂O₃ bulk</th>
<th>0 h treatment with La₂O₃ NP</th>
<th>24 h treatment with La₂O₃ NP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td>2.1×10⁸</td>
<td>TNTC</td>
<td>2.2×10⁸</td>
<td>TNTC</td>
<td>2.3×10⁸</td>
<td>1.0×10⁷</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>2.8×10⁸</td>
<td>TNTC</td>
<td>2.8×10⁸</td>
<td>TNTC</td>
<td>2.7×10⁸</td>
<td>2.8×10⁷</td>
</tr>
<tr>
<td>Experiment 3</td>
<td>2.9×10⁸</td>
<td>TNTC</td>
<td>2.8×10⁸</td>
<td>TNTC</td>
<td>2.8×10⁸</td>
<td>1.7×10⁷</td>
</tr>
<tr>
<td>Mean</td>
<td>2.6×10⁸</td>
<td>NA</td>
<td>2.6×10⁸</td>
<td>NA</td>
<td>2.6×10⁸</td>
<td>1.8×10⁷</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>0.4×10⁸</td>
<td>NA</td>
<td>0.3×10⁸</td>
<td>NA</td>
<td>0.3×10⁸</td>
<td>0.9×10⁷</td>
</tr>
</tbody>
</table>

* TNTC—too numerous to count; ** NA—not applicable

2.2.3 Quantitative evaluation of pyocyanin production

Interestingly, treatment of *P. aeruginosa* cultures with La₂O₃ bulk material resulted in a significant increase in the significant reduction in the growth of *E. coli* and *P. aeruginosa* were observed and the results are not presented here.

Bactericidal properties of La₂O₃ NPs were compared with positive control silver nanoparticles (Ag NP) and found to be nil growth. The results suggested that La₂O₃ NPs did not show the same effectiveness and spectrum of antibacterial properties as seen with positive control Ag NP. Ag NP exhibited strong antibacterial activity against all pathogens tested. There are a number of studies reporting the antimicrobial activities of Ag NP. Some hypothesis that were proposed antibacterial mechanism of Ag NP, are free radicals released by the Ag NP that could attack the cell wall of bacteria and penetration of Ag NP into the cell and causing cell death[9,29].
production of pyocyanin pigment. This effect was not seen with La$_2$O$_3$ NP. The pigment produced by the *P. aeruginosa* in the presence of La$_2$O$_3$ bulk material was confirmed as pyocyanin by treating it with chloroform and hydrochloric acid, which gave its characteristic colour. La$_2$O$_3$ bulk treated *Pseudomonas* produced comparatively much higher concentrations of the pyocyanin compared to both untreated control and La$_2$O$_3$ NP treated for 24 h of treatment. The amount of pyocyanin production at 0 and 24 h of treatment are given in Table 2. The exact biological significance of this phenomenon is not fully understood.

### 3 Conclusions

In conclusion, we showed that La$_2$O$_3$ NPs had antibacterial activity against *S. aureus* and possibly against other gram-positive bacteria. Further research needed to identify the exact mechanism of bactericidal effect is still underway.

### References:


