Antimicrobial activity of aluminium oxide nanoparticles for potential clinical applications

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The last two decades have seen a drastic increase in bacterial resistance to antibiotics and is a major concern for medical professionals world-wide. Metal oxide nanomaterials are currently the most promising tools applied as antimicrobial agents for diagnosis of diseases, drug delivery systems, sun screens and ceramics in biomedical and pharmaceutical arena. The antimicrobial activity of metal oxide nanoparticles is due to the large surface area which ensures a broad range of reactions with bio-organics present on the cell surface. Alumina nanoparticles have immense commercial applications and are being studied for their antimicrobial behaviour. At near-neutral pH, an electrostatic interaction plays a possible role in toxicity of alumina nanoparticles, due to interaction between its positive surface charge and negatively charged bacterial cells leading to NP adhesion onto bacterial surfaces and decrease in cell viability. This review attempts to elaborate the antimicrobial behaviour of alumina nanoparticles and its potential clinical applications.

Keywords: alumina; antimicrobial; clinical

1. Introduction

Infectious diseases were one of the major causes of mortality till the late 19th century [1] until the discovery of antibiotics - the first being penicillin whose commercial production commenced around 1940 [2]. Antimicrobial agents are of high relevance in numerous commercial applications such as in packaging industries, environmental, textiles and medical products to name a few [3]. However indiscriminate use of antibiotics has led to bacterial resistance to the antimicrobial drugs thereby triggering a greater need for efficient antimicrobial agents to which bacteria might not develop resistance. Nanoparticles with their large surface area to volume ratio have been studied to be likely candidates for antimicrobial agents. The antibacterial activity has been observed to vary as a function of surface area in contact with the microbe; therefore nanoparticles with large surface area ensure a broad range of reactions with the bacterial surface [4]. Microbes are more unlikely to develop resistance against nanoparticles since they attack a broad range of targets which requires the microorganism to simultaneously undergo a series of mutations in order to protect themselves [5]. Metal oxide nanoparticles have immense applications in numerous fields ranging from water treatment, cosmetics, medicine and engineering to name a few [6]. Though the antimicrobial behaviour of metal nanoparticles such as silver have been widely reviewed, to the best of our knowledge there are not much significant reviews on the antimicrobial properties of metal oxide nanoparticles with alumina in particular.

Therefore this review has been designed to discuss the antimicrobial behaviour of alumina nanoparticles and its possible clinical applications. The review has been organised into four sections. In the second section, we discuss in brief about the era of antibiotics and their drawbacks, while in section three the commercial applications and antimicrobial activity of metal oxide nanoparticles are discussed in general. The next section deals with the antimicrobial studies of alumina nanoparticles and possible clinical applications in detail while the last section discusses the future perspectives in this emerging field.

2. Use of antibiotics: end of an era?

The late Victorian period witnessed a number of observations on microbial antagonism which was the ability of one microorganism to kill or limit the growth of another [7]. But the most famous observation came in 1928 when Alexander Fleming discovered Penicillin, an antibiotic produced by Penicillium mould against Staphylococcus aureus [8]. But the irrational and indiscriminate use of antibiotics in agriculture and to treat common infections ultimately led to the problem of antibiotic resistance. For example rampant use of methicillin has led to the development of Methicillin Resistant Staphylococcus aureus (MRSA) which is still a major concern in hospitals [9] whereas indiscriminate use of third generation antibiotics such as Vancomycin and Cephalosporin has led to new strains of Vancomycin resistant Enterococcus [10].

It has been observed that corrective measures such as optimising the use of antibiotics might not decrease instances of antibiotic resistance in the near future [9]. Therefore these drawbacks led scientists to focus on developing antimicrobial agents to which microorganisms might not develop resistance. Thus came metal oxide nanoparticles into limelight.
3. Metal oxide nanoparticles

3. a An overview

Before the commercial usage of nanoparticles, ultrafine particle pollution was an unavoidable byproduct of industrial revolution and received tremendous attention due to its adverse effects on human health. However, it was only a few decades ago that scientists discovered that the beneficial properties of these ultrafine particles (e.g. improved hardness, special optic and magnetic properties) which could be commercially exploited thus leading to the large scale production of nanoparticles. Metal oxide nanoparticles have been recently manufactured at the industrial level and have tremendous applications in water treatment, medicine and cosmetics to name a few. These materials are present in a number of commercially available products including filters, catalysts and many other industrial applications. Titania nanoparticles are widely used for protection against UV ray exposure due to their high refractive index. Many sunscreens contain these nanoparticles as well as surface coating products which are colorless and reflect UV rays more efficiently than larger particles [11]. Nanosized aluminium containing particles are also used in industrial, medical products and in energetic systems (composite propellants) to replace lead primers in artillery, etc. For example, aluminum nanoparticles are used in explosive combinations [12] and titanium dioxide nanoparticles are mostly used as photocatalysts and adsorbents in consumer products like in sunscreens and as catalysts in sterilization and chemical engineering [13-15].

Nanomaterials are ideal forms of antimicrobial agents since 40 to 50% of the molecules or atoms present on the surface of particles will react uniquely to the targeted species and also have large surface area thus surface reactivity is relatively higher in comparison to bulk materials. However, chemical composition and physical dimensions govern the specific type of nano-bio interaction that is characteristic for each type of nanomaterials. Since the physico-chemical characteristics of nanoparticle play a significant role in determining its antimicrobial action, we discuss in brief some of the salient physico-chemical properties of metal oxide nanoparticles in the following section.

3. b Physico-chemical characteristics

Metal oxide nanoparticles (NPs) can be composed of a variety of materials, including titanium, zinc, cerium, aluminum and iron oxides [16]. Nanoparticles possess different chemical properties when compared to bulk types of similar chemical composition [17]. Furthermore, the size of such particles is one of the major causes responsible for the changes in their fundamental physical and chemical properties yielding completely new and different physico-chemical properties. For example, titanium dioxide loses its white color and become colorless at decreasing size (<50 nm) [17].

Metal oxide based engineered nanoparticles (ENPs) are usually coated with inorganic or organic compounds, such as citrate, cysteine, carbonate or surfactants such as sodium dodecyl sulfate to augment the stability of the colloidal suspension [18]. However, the surface properties of ENPs in aqueous suspensions are strongly dependent on the composition of these coatings, which results, at neutral pH, in a surface charge of the ENPs, which then results in their stabilization with respect to aggregation [19]. Furthermore, the surface properties of metal oxide NPs are also determined by their acidity constants and zero-point of charge [20, 21]. For example, titania nanoparticles are expected to be positively charged at pH 6 and negatively charged at pH 7, whereas, silica nanoparticles are generally negatively charged, as their zero-point of charge is located at around pH 2 [22]. The particle aggregation and deposition are closely related phenomena [23]. Physico-chemical properties play a vital role in metal oxide antimicrobial activity; including particle aggregation, crystal phase and surface modification.

3.1 Antimicrobial activity of metal oxide nanoparticles

As discussed in the previous section, reactive groups on a particle surface are likely to modify its biological activity. Therefore, changes in surface chemistry and the type of metal oxide nanoparticle are important in terms of microbial toxicity issues [17]. In this section we discuss the antimicrobial behaviour of metal oxide nanoparticles with the exception of alumina, which is discussed in detail in the next section.

Inorganic metal oxide nanoparticles can be used as effective disinfectants in view of their non toxic profile, stability and antibiotic properties [24]. The antibacterial activity of zinc oxide nanoparticles towards Escherichia coli was studied by Brayner et al [25] and the oxide nanoparticle was observed to disrupt the membrane structure of the gram negative organism. Zinc oxide nanoparticles are commonly used in antibacterial formulations. But there are instances in which the zinc oxide nanoparticles in aqueous media tend to aggregate into large flocculates due to their hydrophobic nature thus inefficiently interacting with the microorganism. In view of this drawback, Gordon et al. [24] combined zinc oxide with iron oxide to produce magnetic composite nanoparticles with improved colloidal stability and effective antibacterial activity against Staphylococcus aureus and E. coli.

In another study, cobalt doped zinc oxide nanoparticles were prepared by co-precipitation method and were observed to have notable antibacterial activity against E. coli, Klebsiella pneumoniae, Shigella dysenteriae, Salmonella typhi,
Pseudomonas aeruginosa, Bacillus subtilis and S. aureus [26]. Premanathan et al. [27] performed an interesting study on antibacterial activity of zinc oxide nanoparticles in which they concluded that the nanoparticles had a more pronounced effect on gram positive organism namely S. aureus when compared with gram negative organism namely E. coli and P. aeruginosa. Zinc oxide nanoparticles have also been observed to possess significant antifungal activity against Fusarium sp. in a concentration dependent manner [28].

Positively charged cerium oxide nanoparticles has been observed to bind to gram negative cell membrane by electrostatic attraction [29] thus indicating that charge of medium and the surface of the microorganism play a major role in determining its activity. Silva-Ag nanoparticles have also been prepared by γ irradiation method and their antibacterial and antifungal activity studied. The study showed that the nanoparticles had significant activity against S. enterica serovar Typhimurium and against the fungi Botrytis cinerea with 99% microbial reduction at 50 ppm [30].

The antimicrobial behaviour of titania nanoparticles have been extensively studied over the years [31]. Titania was observed to have the maximum activity against E. coli and minimum activity against the fungi Candida albicans which was related to the complexity of the cell membrane [32]. Titania has been extensively used as a semiconductor photocatalyst which exhibits bactericidal activity in the presence of near UV light and UV-A [33]. The photo catalytic antimicrobial activity was observed by [32] to depend on the membrane thickness and was studied to be highest in virus when compared to bacteria and bacterial spores.

We, in our group, have also done extensive work on the antibacterial activity of these nanoparticles towards E. coli, Pseudomonas aeruginosa and Bacillus subtilis and studied the membrane permeability of the nanoparticles towards the organisms. We observed a concentration dependent growth inhibitory effect on the three bacterial strains [34]. Preliminary studies on the activity of titania nanoparticles against microalgae species have also been conducted for which a concentration dependant decrease in chlorophyll content was observed [35].

ROS production has been observed to be the attributing factor for the antimicrobial activity of titania nanoparticles for which the microbial surface is the primary target [36]. Microorganisms resistant to UV radiation and desiccation have been studied to be inactivated by titania nanoparticles [37].

The significant antimicrobial activity of the above discussed metal oxide nanoparticles has enabled their specific applications such as using zinc oxide nanoparticles in drug carriers, medical filling materials [38,39] and in textiles [40] to name a few. Titania nanoparticles for example are used for water treatment [41], in food industry [37] and in orthodontic appliances [36].

The following section is entirely concerned with our core area of discussion, namely the antimicrobial properties of alumina nanoparticles and their potential clinical applications since these nanoparticles are currently one of the two US market leaders for nanosized materials [42].

### 4. Alumina nanoparticles: an overview

Alumina nanoparticles are thermodynamically stable particles over a wide temperature range. They are corundum like structure with oxygen atoms adopting hexagonal close packing with alumina ions filling two thirds of the octahedral sites in the lattice [3]. Murdock et al. [43] have observed that the particle behaviour was also influenced by particle size, shape and surface charge. Nanoparticles tend to aggregate in hard water and seawater due to particle interaction with organic matter present in water. Aggregations of particle are also influenced by pH and salinity, which state the dispersion ability of particles in the suspension that lead to alter toxicity assessment. Before implementing toxicity studies certain important parameters have to be taken into consideration such as particle size, size distribution, morphology, composition, surface area, surface chemistry and particle reactivity in solution which need to be accurately characterized as prerequisites.

#### 4.1 Antibacterial behaviour of alumina nanoparticles

Recently Bala et al. [44] synthesized alumina-silver composite nanoparticles by a simple, reproducible, wet chemical method, with the surface of the oxides modified with oleic acid. Preliminary antibacterial studies performed using disc diffusion assays against E. coli DH5α and Staphylococcus epidermidis NCIMB 12721 suggested that the composite nanomaterials have immense potential as antimicrobial agents.

We at our research group have carried out extensive studies on the antibacterial behaviour of these nanoparticles. We investigated the antibacterial effect of alumina towards E. coli and growth inhibition was studied at varying concentrations of alumina. To ascertain whether the antibacterial activity was due to particles in the broth or due to the specific interactions with bacterial cellular components, a growth reversibility study was performed. We observed a negligible dependence of growth rate with the concentration of the nanoparticles. The growth reversibility studies indicated a significant retardation in growth of recultured E. coli cells which had prior exposure to the nanoparticles. A decrease in the extracellular protein content after nanoparticle exposure was also observed as indicated by protein assays and FTIR studies. The results indicated that alumina nanoparticles had a mild inhibitory effect against E. coli at high concentrations up to 1000 µg/ml [3]. Figure 1a, 1b and 1c shows the scanning electron microscopic images of nano alumina interacted cells of E. co, P. aeruginosa and B. subtilis. The SEM images clearly indicated the change in cell
shape and agglomerated particles on the cell wall. Arrow mark indicated the change of cell wall morphology of bacterial cells. The alumina interacted cells shows distorted cell morphology, indicating the distortion on bacterial cells (unpublished data, Mukherjee et al., 2011).

Fig. 1 Scanning electric micrograph of alumina nanoparticles interacted (a) E. coli (b) P. aeruginosa (c) B. subtilis (unpublished data, Mukherjee et al., 2011)

4.1.1 Comparison of antibacterial activity of nano and bulk counterparts

Since nano metal oxides have greater surface area than their bulk counterparts [45], it is expected that they might also behave in a different way on interaction with microorganisms. Few studies have compared the difference in toxicity between nano and bulk alumina. In a significant study, the activity of nanoscale alumina against B. subtilis, E. coli and Pseudomonas fluorescens was examined and compared with its bulk counterpart. The nanoparticle had higher toxicity than bulk at the concentration studied and it was observed that P. fluorescens was the most sensitive [45]. Similar results were also obtained by Balasubhramanyam et al. [46] who observed that Salmonella sp. had more mutagenicity towards alumina nanoparticles when compared to the bulk.

4.1.2 Antibacterial activity due to released ions

The antimicrobial activity of alumina nanoparticles due to the release of metal ions has also been addressed in a few studies. In their study, Jiang et al. [45] observed that dissolved metal ions were not present in a measurable quantity in the supernatant of the suspension thus ruling out the role of aluminum ions in nanoparticle mediated toxicity. They observed that the nanoparticles attached to the surface of the bacteria due to surface charge; bacterial surface was negative while alumina nanoparticles were positive at the pH studied.

Since there are only a few studies on the role of aluminium ions on bacterial toxicity, we have undertaken a study in which we isolated B. subtilis from aluminium contaminated site (thereby the bacteria isolated was aluminium tolerant) and studied its growth rate against nano and bulk alumina. We studied the interaction of the isolated bacteria with varying concentrations of bulk, nano alumina along with an equivalent concentration of aluminium ions. We observed that the bacterial toxicity was nearly the same between the nano form and the equivalent salt concentration. However the antibacterial activity of the bulk was significantly lesser than the nano form. More detailed studies are being carried out.

4.2 Antimicrobial activity of alumina nanoparticles to other microbial forms

The toxicity of alumina nanoparticles to microalgae has been studied recently by Sadiq et al. [35]. They investigated the difference in toxic response of micron sized and nano sized alumina towards Scenedesmus sp. and Chlorella sp. A growth inhibitory effect of the nanoparticle was observed against both the species and an evident decrease in the chlorophyll content was also observed in the cells treated with nanoparticles. An interaction of the nanoparticles with the cell surface was suggested as the possible mechanism for the toxicity.

The notable antimicrobial activity of alumina nanoparticles would enable their use in a number of clinical applications and is detailed in the following section.
5. Use of alumina nanoparticles in potential clinical applications

5.a In membranes
Polymeric membranes have been widely investigated for their performances in drug delivery [47]. Recently polyethersulfone/ aluminium oxide membranes with higher porosity have been developed for drug delivery applications [48]. Another notable study observed that the addition of alumina nanoparticles to PVDF membranes led to the effective improvement of the membrane performance. The study observed that with increased alumina concentrations, the water permeate fluxes, mechanical properties as well as the hydrophilicity increased [49]. The authors also reported that the addition of alumina nanoparticles to the polymeric membranes needed to be carried out with utmost care since excessive addition could lead to decrease in membrane strength.

5.b Drug delivery
Most of the drugs are delivered into the body predominantly using the oral or intravenous route. However other strategies need to be adopted to deliver drugs containing biological agents such as proteins [50] and this is when nanoparticles come into play. Alumina nanoparticles are also considered for drug delivery applications due to their potential scavenging behaviour [51]. The scavenging property has been related to their ability to act as direct antioxidants, block ROS production and also cause a reduction in ROS production [52].

5.c Orthopaedics
Ceramic nanocomposites are being considered as potential third generation orthopaedic biomaterials in view of their ability to match the chemical, biological and the mechanical properties of natural bone [51]. The material that is to be used in orthopaedics should possess high fracture resistance, ductility and weight to strength ratio [53].

The application of aluminium oxide nanoparticles in the medical field is still in the process of gaining momentum and we can expect a major role of metal oxide nanoparticles in this field in the years to come.

6. The road ahead
Though there is immense potential available for alumina nanoparticles for the detection of infections, delivery of antimicrobial drugs and regarding its role in overcoming antibiotic resistant pathogens there a number of challenges that need to be tackled in order to facilitate its smooth application.

Even though alumina nanoparticles have been established as potential antimicrobials there are a few challenges which need to be efficiently tackled before its use in clinical applications. In depth studies regarding the interaction of the nanoparticles with cells, tissues and organs as well as the optimum dose required to produce therapeutic effects [2] need to be ascertained before we can expect a more meaningful role of the metal oxide nanoparticles.

Drugs are generally administered using oral and intravenous routes however it usually leads to undesirable effects in non target sites. Therefore local administration of drugs using nanoparticles appears as an ideal choice. Nanoparticles formulated for dispersing water insoluble drugs, optimised low density microstructures for specialised drug delivery to lungs [54]. Detailed studies regarding high local drug concentration should be carried out.

7. Concluding remarks
Antibiotics have been used for more than half a century to treat infectious diseases. However antibiotic resistance acquired by the microorganisms came as a major blow to the medical fraternity. Recently the use of nanoparticles are being explored as potential alternatives as antimicrobial agents as the microorganisms are unable to develop resistance against nanoparticles. Notable antimicrobial properties of aluminium oxide nanoparticles are enabling their application in the clinical sector. However detailed studies regarding the interaction of alumina nanoparticles with cells need to be ascertained before extensive use in medical application.

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