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The contribution of zinc ions to the antimicrobial activity of zinc oxide

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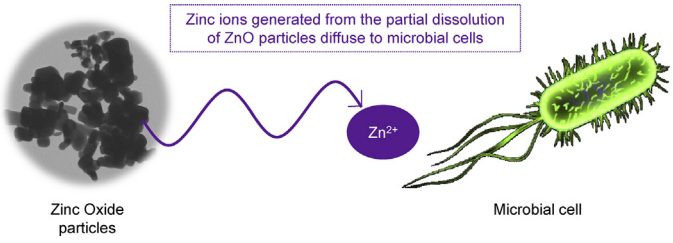
HIGHLIGHTS

- Proteins contained in a broth medium increase the solubility of ZnO particles.
- The respective activities of ZnO and Zn^{2+} show specificity with respect to the microorganisms.
- The contribution of Zn^{2+} to the antimicrobial activity of ZnO depends on the strain.
- The dissolution process depends on time, ZnO concentration, and type of ZnO powder.
- The combination of the three antimicrobial mechanisms of ZnO is beneficial.

GRAPHICAL ABSTRACT



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ABSTRACT

Zinc ions (Zn^{2+}) exhibit antimicrobial activity against various bacterial and fungal strains. The partial dissolution of zinc oxide (ZnO) particles releases Zn^{2+} ions in aqueous suspension that contributes to the antimicrobial activity of ZnO. In addition to the activity of the soluble zinc species that is common with water-soluble zinc salts, ZnO combines two additional mechanisms of antimicrobial activity that supplement its activity as preservative in topical formulations: generation of reactive oxygen species and by direct contact to the cells walls. The present study aims at the evaluation of the contribution of the soluble zinc species to the antimicrobial activity of ZnO on microbial cultures in broth medium and the investigation of the dissolution of zinc from ZnO suspensions. The antimicrobial activities against the five microorganisms of the Challenge Tests were measured for suspensions of three ZnO grades in broth, and for the isolated liquid phase of the suspensions containing soluble zinc species. The Zn^{2+} released in the broth brought about a significant contribution to the overall antimicrobial activity of ZnO. The complexation of Zn^{2+} ions by the components of the broth increased the solubility of the zinc in the liquid medium. The respective activities of the soluble zinc species and ZnO particles showed specificity with respect to the microbial strains. Dissolution was faster for high concentrations of ZnO and for ZnO powders of larger specific area. Such conditions led to a better antimicrobial efficacy of ZnO powders. ZnO appears an advantageous alternative to soluble zinc salts such as zinc gluconate.

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1. Introduction

Zinc oxide (ZnO) is an efficient antimicrobial agent that acts by means of several mechanisms involving different chemical species. Three distinct mechanisms of action have been put forwards in the literature: (i) the production of reactive oxygen species (ROS) because of the semiconductive properties of ZnO [1,2,3], (ii) the destabilization of microbial membranes upon direct contact of ZnO particles to the cell walls [4–6], and (iii) the intrinsic antimicrobial properties of Zn^{2+} ions released by ZnO in aqueous medium [7–9]. The present study aims at investigating the contribution of the soluble species released by ZnO, especially Zn^{2+}.

Zinc is an essential element for microorganisms and higher organisms because it is involved in many vital cellular reactions at its low endogenous concentrations [10–13]. The concentration of zinc is 10^{-4} M in blood [14]. Optimal rates of this cofactor are needed for catalytic and structural activities [14,15]. Zinc concentration is regulated under physiological conditions by several transporters [15–17], so that Zn^{2+} ions are essentially nontoxic to higher organisms [3]. Homeostasis regulates zinc uptake by cells, but it does not control zinc adsorption to cell membranes however. Increase of Zn^{2+} concentrationsabove optimal levels (typically between 10^{-7} M and 10^{-5} M depending on the microbial strain [18]) perturbs Zn^{2+} homeostasis and allows entry of Zn^{2+} inside cells, so that zinc starts being cytotoxic to prokaryotes above a concentration of $\sim 10^{-4}$ M [18,19]. Therefore, Zn^{2+} displays an antimicrobial activity and could act as either antibacterial or antifungal agent. The antimicrobial properties of Zn^{2+} have been known since a long time, both against bacterial [20–22] and fungal strains [23].

According to several reports on water-soluble zinc salts, the antimicrobial activity of Zn^{2+} depends on its concentration and contact duration. Zinc chloride acts in a dose-dependent manner against *Escherichia coli* [21,22]. Zinc acetate exhibits an antibacterial activity on *Pseudomonas aeruginosa* and *Staphylococcus aureus* for zinc concentrations above 11 mmol L^{-1} [20]. Moreover, a prolonged contact of zinc (at 100 $\mu g\, g^{-1}$) to *Aspergillus brasiliensis* spores inhibits their germination by 25% [23]. These antimicrobial activities were explained by two mechanisms, both leading to cell death: (i) a direct interaction with microbial membranes leading to membrane destabilization and enhanced permeability [24]; (ii) an interaction with nucleic acids and deactivation of enzymes of the respiratory system [25].

In dermatological products, zinc ions are interesting biocides and/or antimicrobial preservatives provided that high enough concentrations of Zn^{2+} are generated. The previously mentioned zinc salts can be simply dissolved in the aqueous medium. An alternative is solid powder such as ZnO particles that release Zn^{2+} in the aqueous medium. It is indeed recognized that part of the antimicrobial activity of ZnO particles originates from their ability to partially dissolve in aqueous media [26,27]. Release of Zn^{2+} would contribute to the global antimicrobial properties of this inorganic powder [7,29]. Nevertheless, the contribution of Zn^{2+} to the antimicrobial activity of ZnO is still unclear. Since ZnO particles exhibit two additional antimicrobial mechanisms with respect to zinc salts (ROS and direct contact) the combination of these three types of action broadens the antimicrobial spectrum of ZnO compared to zinc salts.

The present study has been focused on the contribution of zinc cations generated from the partial dissolution of ZnO particles in aqueous media to the global antimicrobial action. Even though the antimicrobial mechanism of Zn^{2+} has been disclosed, the antimicrobial activity of ZnO *via* a contribution of Zn^{2+} is still under debate because of the complexity of the underlying phenomena. According to Sawai [28] and Jiang et al. [6], the contribution of Zn^{2+} to the antimicrobial efficacy of ZnO particles would be minor because too low concentrations of soluble zinc species are released from the dissolution of ZnO particles. In other instances reported in the field of dentistry applications, the contribution of Zn^{2+} is predominant [30,31]. The aim of the present work was the assessment of the contribution of soluble ionic species of zinc to the antimicrobial efficacy of ZnO powders, as well as the factors that influence the dissolution of the particles. Indeed, it has been reported that the dissolution phenomenon was influenced by numerous parameters sorted into two types:

- the chemistry of the environmental media such as the pH [32,33], the duration of exposure [34–36], UV irradiation [32,37], the presence of other substances [33,38] or microorganisms [39–41];
- the physicochemical properties of the particles such as the elementary particle size [35,42,43], their porosity [44], their shape [35], their concentration [45].

The impact of all these parameters is not fully understood.

In order to use ZnO particles as an efficient antimicrobial preservative in cosmetic and dermopharmaceutical products, it was firstly aimed at discriminating the contribution of zinc cations generated by ZnO particles from the overall antimicrobial activity, and secondly identifying the parameters which directly impact the dissolution phenomenon and would enhance this mechanism. The study was performed on the five microbial strains used for Challenge Tests for checking the safety of pharmaceutical and cosmetic products. Microbiological tests on solid agar plate and in broth culture were performed to evaluate the antimicrobial efficacy of both ZnO particles and Zn^{2+}. This work was performed taking into consideration the dissolution of ZnO particles in aqueous media depending on the environmental conditions and on the physicochemical characteristics of ZnO powders using three different ZnO grades.

2. Materials and methods

2.1. Materials

The following ZnO powders of pharmaceutical grade were studied: ZnO-1 from Rockwood Pigments (Beltsville, US); ZnO-2 from SILOX (Engis, Belgium); ZnO-3 from Zinc Corporation of America (Pittsburgh, US). Zinc gluconate (ZnG) was supplied by Seppic (Castres, France).

2.2. ZnO particles characterization studies

The physicochemical properties of the three ZnO grades were investigated as previously reported [46]. The characteristics of the powders were assessed in a dry state by determining their specific area and the porosity by nitrogen adsorption measurements using a Tristar 3000 Micromeritics BET instrument. The specific area was determined by the Brunauer–Emmett–Teller (BET) multipoint method and the pore volume was analyzed by the Barrett–Joyner–Halenda (BJH) method. The crystal structures were established by X-ray diffraction measurements performed at the 'Centre Henri Longchambon' facility (University of Lyon) using a Bruker AXS D8 Advance X-ray diffractometer operating with the Cu Kα1 line at 1.54 Å wavelength. The crystallite size was estimated from the width at half height of the Bragg peaks using the Debye–Scherer equation. The apparent density was studied following an adapted protocol of the European Pharmacopeia. Size and shape of the elementary particles were studied by transmission electron microscopy (TEM) performed at the 'Centre Technologique des Microstructures' facility (University of Lyon) on a Philips CM120 microscope operating at 80 kV acceleration. A dilute aqueous suspension (0.1%) was spread on Formvar/carbon grids and dried before observation.

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