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Zinc Oxide Nanoparticles Dispersed in Ionic Liquids Show High Antimicrobial Efficacy to Skin-Specific Bacteria

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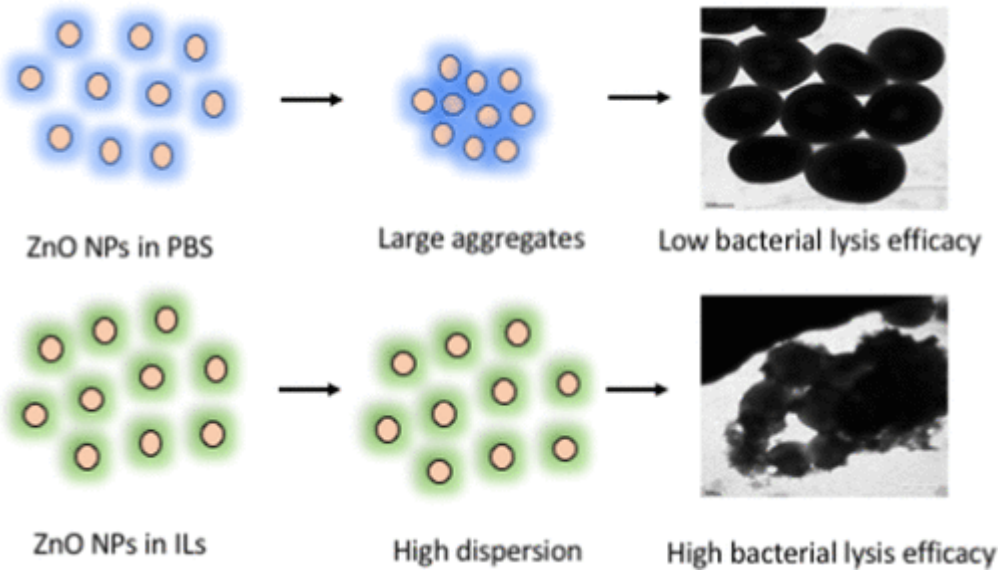
Abstract



Zinc oxide (ZnO) nanoparticles have been shown in the literature to have antibacterial properties and have been widely used in antibacterial formulations. However, one of the problems with ZnO nanoparticles is their tendency to aggregate, thereby causing damage to normal cells and lowering their antibacterial efficacy during application. In this work, we have attempted to avoid this by using a combination of ZnO nanoparticles and ionic liquids, a class of low melting salts containing organic cations and organic/inorganic anions that show antibacterial property as well, and tested the antibacterial activity of this dispersion. ZnO nanoparticles of 60 nm were dispersed in two different ionic liquids—choline acetate (IL1) and 1-butyl-3-methylimidazolium chloride (IL2)—to achieve high dispersibility, whereas ZnO dispersed in phosphate-buffered saline was taken as a control. These dispersions were tested on four strains—*Escherichia coli*, *Bacillus subtilis*, *Klebsiella pneumoniae,* and *Staphylococcus epidermidis*. Maximum efficiency was obtained for ZnO nanoparticles dispersed in imidazolium-based ionic liquids against skin-specific *S.*

epidermidis. Skin infections induced by *S. epidermidis* are prevalent in hospital-acquired diseases. In most cases, traditional antibiotic-based therapies fail to combat such infections. Our strategy of developing a dispersion of ZnO nanoparticles in ionic liquids shows superior antibacterial efficacy in comparison to that shown individually by ZnO nanoparticles or ionic liquids. We have also established that the mechanism of killing this skin-specific bacterium is possibly through the production of reactive oxygen species leading to bacterial cell lysis. Further, we showed that this formulation is biocompatible and nontoxic to normal keratinocyte cells even under coculture conditions.

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Supporting information for publication

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1. Materials and methods:

1.1 Materials:

Trypticase soy broth, media components, crystal violet, Propidium Iodide (PI) stain, Zinquin ethyl ester dye, all the media components and other chemicals were obtained from Sigma Aldrich.

1.2. Methods

1.2.1 Zinquin assay

Dried powder of ZnO nanoparticles was dispersed in Phosphate buffer saline (PBS) or in IL%1 and IL%2 at a concentration of 40 µ

g/ml. The solution was sonicated for 10 minutes (pu
lse on: 50
seconds and pulse off: 10 seconds cycle) for proper dispersion. Next, the solution was
centrifuged at 8000 r.p.m at different time points
(0,2,4,8 hrs). 100
µ
l of supernatant was mixed
with 25
µ
M of zinquin ethyl ester dye (Ex 364 nm/Em 385 nm)
in order to quantitate Zn^{2+} in the
solution. Fluorescence reading was captured in a Te
can plate reader.

1.2.2 Bacterial Live/Dead assay

Bacterial viability test was performed as per the p
rocedure mentioned in the Baclight™
Live/Dead assay kit (Thermo Fisher Scientific). In
brief, 30 mL culture of
S. epidermidis
was
grown to late log phase in LB media. Treatment was done with 105
µ
g/ml of ZnO+PBS,
ZnO+IL2 (105
µ
g/ml ZnO in 10mM IL2), 10 mM of IL2 and 100% alcoho
l. The mixture was
concentrated at 10,000 × g for 10–15 minutes. Super
natant was removed and resuspended in the
pellet in 2 mL of 0.85% NaCl. To this 1.5 Dl of PI was added and mixed thoroughly. The
suspension was incubated in the dark for 15 minutes
. The stained samples were analyzed through
both Flow cytometer FACS%Accuri
™
(Becton Dickinson, USA) using BD Accuri
™
software
and fluorescence microscope Leica TCS SP8. Average of three independent experiments was
considered.

1.2.3 Biofilm inhibition assay