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Article

Surface cleanliness of hydrothermally grown zinc oxide microparticles compared to commercial nanoparticles

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SUMMARY

Zinc oxide (ZnO) nanoparticles are attractive candidates for application as antibacterial agents due to their effectiveness against antibiotic-resistant strains of both gram-positive and gram-negative bacteria. Despite this potential, applications are limited by fundamental gaps in current understanding of their underlying antibacterial pathways. ZnO microparticles are less often used in antibacterial research compared to ZnO nanoparticles due to the potential of nanoparticles for internalization into bacterial cells. Microparticles are nevertheless of interest as a research platform as their increased scale allows both the nonpolar and polar faces of the ZnO crystals to be distinguished. This in turn provides a useful platform to study surface interactions with bacteria, allowing for more targeted investigation of antibacterial mechanisms. Previous preliminary studies have indicated that hydrothermally grown ZnO microparticles exhibit comparable antibacterial activity to commercial ZnO nanoparticles further adding to their utility. The purpose of this research was to examine the surface cleanliness of ZnO microparticles in comparison to nanoparticles utilizing both scanning electron microscopy (SEM) as well as Fourier transform infrared spectroscopy (FTIR). The results of our experiments supported our hypothesis that there were no significant differences in the surface contamination of ZnO microparticles compared to nanoparticles. This supports the usage of ZnO microparticles as a viable platform for studying antibacterial mechanisms observed at the nanoscale knowing that in the absence of internalization effects the mechanism of antibacterial action across scales is intrinsic to ZnO and not a result of differing surface cleanliness.

INTRODUCTION

Zinc oxide (ZnO) has distinctive properties that enable it and its composites to act as antibacterial agents via a number of proposed pathways (1). For example, ZnO can dissociate into Zn^{2+} ions which have antibacterial effects within an organism (1). ZnO particle adsorption to the cellular biomembranes can generate abundant reactive oxygen species, which play an essential role in antibacterial functions through oxidative damage to DNA and bacterial membrane (1, 2). Furthermore, ZnO particles can inhibit a wide range of key metabolites involved in central metabolism, including cofactors synthesis, amino acid and organic acid biosynthesis, purines and pyrimidines, and nucleoside and nucleotide biosynthetic pathways (1, 3). This prevents intracellular reactions from taking place, and eventually causes the cell processes to halt. These multifaceted pathways that ZnO can act through make it more versatile than other oxides, such as silver oxide (Ag_2O) or titanium dioxide (TiO2), which can only inhibit bacterial growth through a few of these pathways (4).

However, beyond these proposed pathways, the fundamental physical and chemical mechanisms driving inhibition of bacterial growth by ZnO are still not well described (5). Particularly, the nature of interactions between ZnO surfaces and extracellular material, outside the cell membrane, is not clear. This is important for ZnO microparticles, which, unlike nanoparticles, cannot penetrate into the cell. ZnO microparticles also possess distinct polar hexagonal and non-polar rectangular surface types, which possess differing electrochemical properties that enable varied antibacterial interactions (6). The differences in surface polarity as well as morphology lead to differences in physical and chemical characteristics. As such, a better understanding of surface interactions has the potential to improve the overall understanding of the fundamentals driving ZnO's bacterial growth inhibition.

The purpose of this research was to examine the surface cleanliness of the ZnO microparticles in comparison to the commercial nanoparticles. Surface cleanliness – absence of contaminants at the surface of particles – is critical in how a material (or a layer on a product) interacts with other surfaces, which in turn can affect its functionality. We utilized scanning electron microscopy (SEM) and Fourier transform infrared (FTIR) spectroscopy to measure the surface cleanliness by identifying the nature of surface functional groups present on both ZnO micro- and nanoparticles. SEM and FTIR techniques can confirm the size and shape of these surface functional groups, as well as identify surface structures. Characterizing these structures will enable us to see if there are significant structural differences between ZnO nanoparticle and microparticle surfaces, which may affect their interactions with bacteria or other biological components.

We hypothesized that there would be no significant differences in the surface cleanliness of the microparticles compared to nanoparticles. We synthesized the ZnO microparticles utilizing a bottom-up, hydrothermal growth method and purchased nanoparticles from Sigma Aldrich and ZoChem. The results from the SEM tests showed the relatively high homogeneity of the larger microparticle crystals and their well-defined polar and non-polar faces in contrast to the nanoparticles. The FTIR results showed no significant differences in the surface contamination of ZnO nanoparticles and synthesized microparticles. These results

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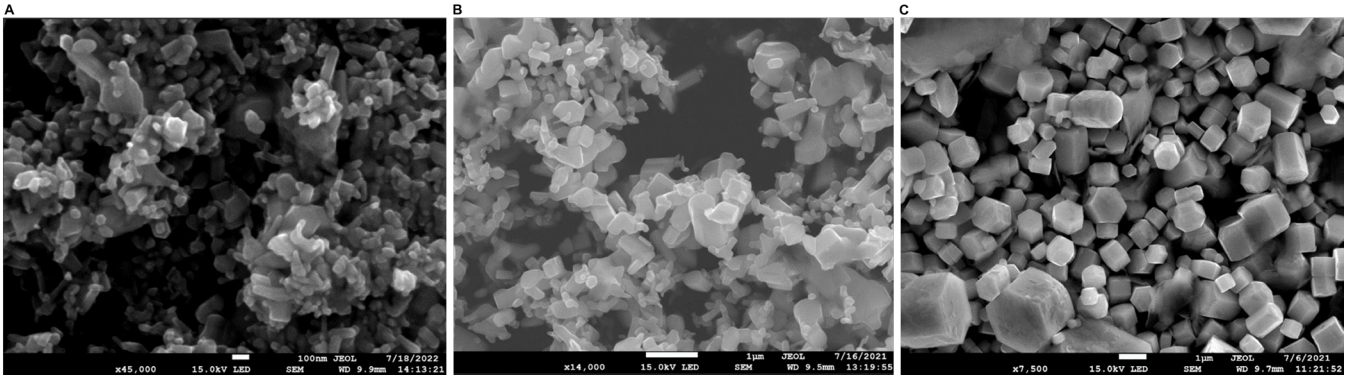

Figure 1: SEM images comparing nanoparticles from Sigma Aldrich, ZoChem, and hydrothermally grown ZnO microparticles. A) SEM image of Sigma Aldrich nanoparticles depicts heterogenous sizes and shapes. Polar and non-polar surfaces are not evident. B) SEM image of ZoChem nanoparticles depict heterogenous sizes and shapes. Polar and non-polar surfaces are not evident. C) SEM image of hydrothermally grown ZnO microparticles depict well-defined hexagonal polar and rectangular non-polar surfaces as well as their homogeneity. SEM was performed utilizing a JEOL FE-SEM instrument at an operating voltage of 15.0kV with a filament current of 84.9μA. Images have magnification labeled as (A) 45,000x with a 100nm scale bar and working distance (WD) of 9.9mm, (B) 14,000x with a 1μm scale bar and WD of 9.5mm, and (C) 7,500x with a 1μm scale bar and WD of 9.7mm..

support the usage of ZnO microparticles as a viable platform for studying antibacterial mechanisms observed at the nanoscale.

RESULTS

To study the surface cleanliness of ZnO micro- and nanoparticles, we utilized SEM and FTIR techniques. The SEM images for nanoparticles from Sigma Aldrich and ZoChem show the heterogeneity of the sizes and shapes of nanoparticles (**Figure 1A, B**). The polar and nonpolar surfaces of these crystals cannot be ascertained. In contrast, SEM results for hydrothermally grown microparticles demonstrate the homogeneity of the microparticles' sizes and shapes barring a few outliers (**Figure 1C**). The hexagonal polar and rectangular nonpolar faces of the microparticles are well-defined.

Analysis of the SEM images of the microparticles with ImageJ software yielded an average surface area for polar hexagonal faces of 1.08 ± 0.44 μm^2 (mean ± SD) while the nonpolar rectangular faces have an average surface of 0.52 ± 0.21 μm^2 (**Figure 1C**). The overall size distribution of the microparticles was narrow indicating that the synthesized microparticles are uniform in size. This is in contrast to the nanoparticle crystals which are heterogeneous with an average surface area of 0.01 ± 0.02 μm^2 and do not have well-defined structures (**Figure 1A, B**).

We conducted FTIR tests to characterize the functional groups at the surface of the ZnO micro- and nanoparticles. Each peak in the FTIR absorbance graphs of the ZnO samples represents a specific functional group as different groups have unique energy signatures (**Figure 2**). For example, in the range 2400-2500 cm^{-1}, there is a large peak, which is assigned to carbon dioxide (O=C=O). The nanoparticles from both Sigma Aldrich and ZoChem displayed practically identical functional group structures, as indicated by their overlapping absorbance spectra. Thus, their surface cleanliness is very similar. The hydrothermally grown microparticles exhibit a similar absorbance graph compared to the nanoparticles but display a lower absorbance rate (**Figure 2**). This discrepancy in the lower absorbance rate can be simply explained by size effects of the larger-sized microparticles vs smaller-sized nanoparticles. Both micro- and nanoparticles showed strong absorption peak around 500 cm^{-1} confirming current literature for ZnO's wurtzite structure (7). The primary peaks of interest are present in both ZnO micro- and nanoparticle samples; these are C-H, C=C=C, O=C=O, and C≡C groups. These peaks are expected due to the presence of organic material in the growth environment. The presence of such groups confirms that our hydrothermal growth method does not introduce surface contaminants different from those of nanoparticles.

The only difference in the FTIR absorbance graphs between ZnO micro- and nanoparticles was a benign O-H

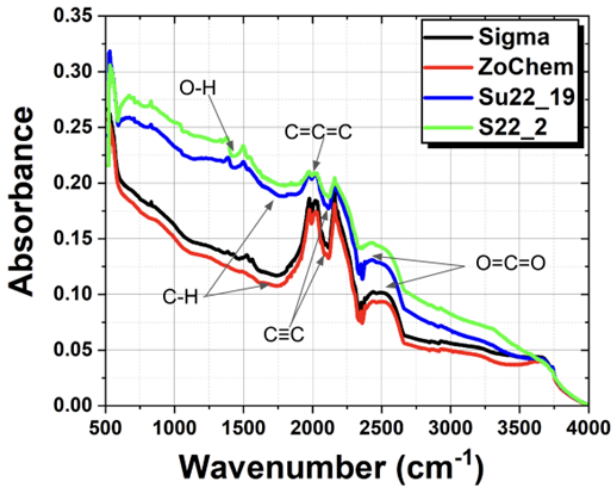

Figure 2: FTIR absorbance spectra for hydrothermally grown ZnO microparticles (Su22_19 and S22_2) show similar peak structure to commercial nanoparticles (Sigma and ZoChem), indicating there are no meaningful differences in surface functional groups. The primary peaks of interest, C-H, C=C=C, O=C=O, and C≡C, are present in both ZnO micro- and nanoparticle samples. The only difference was a benign O-H group for ZnO microparticles which we attribute to water contamination and consider to be inconsequential. ATR-FTIR tests were conducted with a Nexus 670 FT-IR with a KBr beam splitter and purged with dried, CO2-free air.

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group at the 1400 cm-1 region of the ZnO microparticle absorbance spectrum. We attribute this hydroxyl group to contamination of water at the surface during the hydrothermal growth process of microparticles. It is unlikely that this peak is related to amine groups that are active in the 1400 cm-1 region since other peaks attributable to amines are absent, notably in the 1400 – 1600 cm^{-1} region. As such, we consider this O-H peak to be inconsequential to ZnO's antibacterial mechanisms.

DISCUSSION

ZnO microparticles are less often used in antibacterial research compared to ZnO nanoparticles. But microparticles are of interest as their increased scale provides a useful platform to study surface antibacterial interactions thus improving the overall understanding of the physical and chemical mechanisms driving ZnO antibacterial activity. Our research question was whether there were any significant differences in the surface cleanliness of ZnO microparticles compared to nanoparticles that could limit their adoption as a viable platform for studying antibacterial mechanisms observed at the nanoscale.

The SEM images of the ZnO particles showed that the microparticles had much more distinct polar and nonpolar faces compared to nanoparticles. The microparticle crystals exhibited relatively high homogeneity and well-defined polar and nonpolar faces vs nanoparticles. The dependable morphology and abundance of high-quality polar and nonpolar surfaces of the microparticles may assist in better identifying the point of interaction with bacteria allowing for more productive research into ZnO's antibacterial mechanisms.

The similarity of the peak structure in the FTIR spectra across the microparticles and nanoparticles supports the hypothesis that there are no meaningful differences in the surface contamination of ZnO nanoparticles and microparticles. This similarity is desirable as it further verifies the usage of ZnO microparticles as a viable platform for studying antibacterial mechanisms observed at the nanoscale. These peaks are of interest as they may help explain the mechanisms behind potential particle solubility or surface interactions relevant to antibacterial properties as these functional groups may serve as potential sites for ligand exchange. We can proceed knowing that in the absence of internalization effects the mechanism of antibacterial action across scales is intrinsic to ZnO and not a result of differing surface contamination.

ZnO microparticles can be the catalyst for massive improvements in antibacterial research and applications. As opposed to nanoparticles, which are currently more widely used, ZnO microparticles provide many potential enhancements such as being cheaper, easier to synthesize and having better inherent properties such as clearly defined polar and non-polar faces. The results presented here further verify the legitimacy of this platform in comparison to the established literature involving nanoparticles. Microparticles are not internalized unlike nanoparticles. While this makes them well suited to conduct research studies, it might limit their applications if cell internalization is necessary for antibacterial activity.

We intend to continue this research by studying surface interactions between ZnO particles and bacterial cells that are driving their antibacterial properties by utilizing large microparticles that avoid internalization. Further research that studies these surface interactions in conjunction with surface photovoltage analysis to evaluate how the polar and nonpolar surface dynamics might be contributing to these antibacterial properties could have immense potential in enhancing current understanding of the fundamental physical and chemical mechanisms driving bacterial growth inhibition of ZnO.

MATERIALS AND METHODS

ZnO Nanoparticles

Commercial ZnO nanoparticles were purchased from Sigma Aldrich (St. Louis, MO, USA) and ZoChem Inc (Dickson, TN, USA).

ZnO Microparticles

ZnO microparticles were synthesized utilizing a bottom-up, hydrothermal growth method, which has been described previously (5). ZnO microcrystals were grown from three starting ingredients – deionized (DI) water and 1 M concentration of hexamethylenetetramine (HMT) were mixed (**Figure 3**). After five minutes of stirring, 1 M concentration of zinc acetate dihydrate was added to the mixture. A small

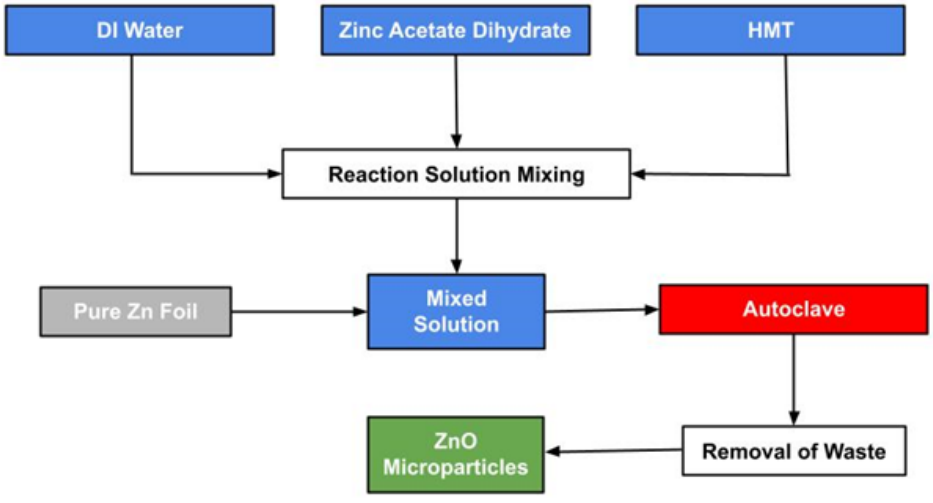

Figure 3: Schematic representation of the hydrothermal method used to synthesize ZnO microparticles.

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piece of 99.999% pure Zn foil is added to the solution as an additional Zn source. This solution was autoclaved at temperatures in excess of 90°C to catalyze the reaction. Next, the resulting solution underwent centrifugation and removal of the supernatant. The remaining solid was cleaned using both DI water and acetone to remove organic residue from the growth process.

Scanning Electron Microscope (SEM)

SEM was performed utilizing a JEOL FE-SEM instrument (JEOL, Peabody, MA, USA) at an operating voltage of 15.0 kV with a filament current of 84.9 μA. SEM is a type of microscope used to produce a magnified image of a sample through the use of electron scattering (8).

Fourier Transform Infrared (FTIR) Spectroscopy

FTIR tests were conducted with a Nexus 670 FT-IR (ThermoFisher Scientific, Waltham, MA, USA) with a KBr beam splitter and purged with dried, CO_2-free air. FTIR spectroscopy is a technique which allows for characterization of functional groups at the surface of a material. Our tests utilized Attenuated Total Reflection (ATR) which is the most common form of FTIR that uses the total internal reflection property of a trapezoidal high refractive index crystal (9). Assignment of peaks present within the resulting spectra were made using standard tables found online in IR Spectrum Table (10).

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