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Zinc release from atomic layer deposited zinc oxide thin films and its antibacterial effect on *Escherichia coli*

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ABSTRACT

Zinc oxide films have been grown by atomic layer deposition (ALD) at different reaction temperatures and in various thicknesses. Zinc-ion release has been examined from the ZnO films in water and in phosphate buffered saline solution (PBS). Additionally, the antibacterial effect has been studied on *Escherichia coli*. The thickness of the ZnO film or its crystal orientation did not affect the rate of dissolution. ALD grown aluminum oxide films were deposited on top of the ZnO films and they acted as an effective barrier against zinc dissolution. The bacteriostatic effect was not dependent on the film thickness but both 45 nm and 280 nm thick ZnO films significantly reduced bacterial attachment and growth in dark conditions by 99.7% and 99.5%, respectively. The results indicated that photoirradiation is not required for to enhance antibacterial properties of inorganic films and that the elution of zinc ions is probably responsible for the antibacterial properties of the ZnO films. The duration of the antibacterial effect of ZnO can be controlled by accurate control of the film thickness, which is a feature of ALD, and the onset of the antibacterial effect can be delayed by a time which can be adjusted by controlling the thickness of the Al_2O_3 blocking layer. This gives the possibility of obtaining dual antibacterial release profiles through a nanolaminate structure of these two materials.

1. Introduction

The field of zinc oxide (ZnO) thin films has been extensively studied and there are several methods such as reactive magnetron sputtering, electron beam evaporation and chemical vapor deposition that have been used in their production [1–4]. During recent years, atomic layer deposition (ALD) has gained immense interest for producing metal oxides and a large number of studies have been conducted on ZnO, for example [5–8]. ZnO in thin film form has been generally found to be an n-type semiconductor with a bandgap of about 3.3 eV, i.e. absorption begins near the UVA area.

ZnO thin films have a great variety of potential applications such as light emitting diodes (LEDs), laser diodes (LDs), solar cells, liquid crystal displays, thin film transistors, and gas sensors [8–12]. ZnO is also applicable to air and water purification and waste remediation [13] due to its photoactive and photocatalytic properties which make it similar in effect to titanium dioxide [7–10,14–17] with a bandgap of 3.2 eV (anatase). In addition ZnO is antibacterial and has already been studied for several decades in antibacterial tests [18–27], and orthopedic and dental clinical treatments [28–30]. A variety of ZnO nanostructures have been studied both in surface energy modifications, photocatalytic treatments, and bactericidal tests [31–34]. Although nanostructures such as powders have been found to be very efficient in use there are risks in their use of causing toxicological and environmental effects [35]. Thin films which are firmly attached to their supports (substrates) may be a good option for air and water purification and antibacterial purposes since they are easily removable from the location where they are utilized. Thin films may be also safer since they probably do not release nanoparticles from their surface.

ALD is a surface controlled gas phase chemical vapor deposition process where thin films can be deposited in a layer by layer manner [36]. The film growth in ALD is self-limiting, contributing several advantages. The thickness of the films can be controlled by the number of reaction cycles, thereby enabling the precise growth of ultra-thin layers. ALD can be used to deposit stoichiometric films with large area uniformity and conformality even on complex surfaces with deformities. Layer-by-layer growth allows one to change the film composition abruptly after each step. This gives the possibility of depositing multicomponent films such as nanolaminates or mixed oxides.

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In this article we have studied the zinc release from ALD -grown zinc oxide thin films which have been immersed in water or body fluid simulant. Zinc release has also been tested with ZnO films which have 5–15 nm of aluminum trioxide (Al_2O_3) deposited on top in order to control the zinc elution from the ZnO film. Al_2O_3 acts as a temporary blocking layer to prevent zinc eluting from the ZnO film. In addition, antibacterial tests have been conducted with ZnO thin films of different thicknesses.

2. Experimental

Zinc oxide films were grown by atomic layer deposition (ALD) using diethylzinc ($Zn(C_2H_5)_2$) (DEZ) and deionized water as precursors at reaction temperatures of 120 °C and 200 °C. The sequence of the deposition was: pulse (DEZ) – rinse (N_2) – pulse (H_2O) – rinse (N_2) with timings of 0.7 – 1.5 – 0.5 – 1.0 s. Different film thicknesses between 45 nm and 280 nm were deposited. An additional series of films at both temperatures was also created where aluminum trioxide (Al_2O_3) films of 5–15 nm were deposited on top of the ZnO films. Trimethylaluminum and deionized water were used at 220° C in Al_2O_3 deposition. Generated nitrogen of 99.999% purity was used as a carrier and purging gas (INMATEC IMT-PN 1150, INMATEC GaseTechnologie GmbH & Co., KG). The deposition was carried out in a TFS-500 ALD reactor (Beneq Oy) at 5×10^2–1×10^3 Pa pressure. The precursors were kept at 20 °C during the deposition. The film thicknesses were measured with a spectroscopic ellipsometer M-2000FI from J.A.Woollam Co., Inc. on co-deposited samples on polished silicon substrates. The structure and crystalline phases of the films were examined by X-ray diffractometry (XRD) (Phillips X'Pert) using CuKα radiation (λ = 1.54 Å). The XRD patterns were acquired with a glancing angle of 0.2° for the incident beam for a range $2\Theta = 25°$–$60°$ with a step size of 0.02° and with Bragg-Brentano geometry. Morphology was studied with a Hitachi S4800 field emission scanning electron microscope (SEM). Zinc release from zinc oxide films was studied by immersing the samples in deionized water and PBS (phosphate buffered saline) solution. The ZnO films were placed in closed plastic containers and immersed in 5 mL of liquid. The samples were kept in dim light for 24 h and the films were removed from the liquid. The same procedure was performed for blank borosilicate samples in order to provide control samples for the measurement. In the long test series, the films were kept in 10 mL water or PBS. Solution containing the eluted material was collected for analysis every 24 h and the sample was immersed in fresh solution. The test was continued for 38 days. The zinc release was analyzed from the elute-containing solution with inductively coupled plasma mass spectrometry (ICP-OES) using an ACTIVA M spectrometer from Horiba Jobin Yvon.

Antimicrobial activity test (non-growing conditions): as ZnO is a photocatalytic semiconductor, antibacterial tests were performed in the dark to separate the photocatalytic from the chemical Zn release -effect. A fresh shake culture of *Escherichia coli* (*E. coli* K-12 wildtype, K-12 DSM 498, ATCC 23716) was prepared in sterile LB medium by shaking at 37 °C and 200 rpm for 18 h. An exponential growing culture was obtained by dilution with sterile LB medium to an absorbance of 0.1 at 600 nm and shaking to an absorbance of 0.5 at 600 nm which correspond to a concentration of 1.0×10^8 CFU mL^{-1} (determined by serial dilutions and plate counting). The bacteria solutions were further adjusted with KH_2PO_4 buffer (0.3 mM, pH 7.2, sterile) to $\sim 2.5 \times 10^5$ CFU mL^{-1}. ZnO films (triplicate, 2.5 cm × 2.5 cm, 6.25 cm^2) were submerged in 7 mL bacteria dilution. Uncoated borosilicate glass (2.5 cm × 2.5 cm, 6.25 cm^2) served as control surfaces. Samples were incubated on a horizontal shaker (Innova 44, New Brunswick Scientific) at 37 °C and 150 rpm for 1 h. The bacteria concentration were determined at time T_0 and T_{1h} by serial dilutions in saline (undiluted, 1:25, 1:125), plating of an 50 μL aliquot on agar plates and incubation for 24 h at 37 °C to give an estimate of viable cell count as CFU mL^{-1}. The mean value and standard deviation (SD) was calculated from the serial dilutions of each sample). The percent of bacterial reduction was calculated from treated sample (A) directly compared to untreated control (B) at T_{1h} where reduction is defined by R, % (CFU mL^{-1}) $= (B_{\text{CFUmL}^{-1}} - A_{\text{CFUmL}^{-1}})/B_{\text{CFUmL}^{-1}} \times 100$.

Bacterial attachment: a fresh shake culture of *E. coli* was prepared as described above and the bacteria were diluted with minimal LB medium to 1.5×10^6 CFU mL^{-1}. Samples (2.5 cm × 2.5 cm, 6.25 cm^2) and silicon wafer control were placed in a 6-well culture plate (Greiner, Germany) and submerged with 2 mL bacteria dilution, covered with a lid, sealed with parafilm and incubated on a horizontal shaker (Innova 44, New Brunswick Scientific) at 37 °C for 24 h. Then the samples were placed in a fresh 6 well culture plate and washed 4 times with saline. Bacteria attaching to the films were stained with Live/Dead BacLight Bacterial Viability Kit (Life Technologies, Germany) according to the supplier's protocol. The bacteria were imaged on an inverted microscope (Olympus IX-70) equipped with a 100 W mercury lamp, a 20× phase contrast objective and a CCD camera (F-View, Olympus) for digital imaging. Living bacteria stained with SYTO 9 (green) were excited with a band pass filter at 470 nm to 490 nm and the emission was detected by 520 nm. Dead bacteria cells stained with propidium iodide were excited with a band pass filter at 530–550 nm and the emission was detected by 590 nm. Image processing performed with CellSens Dimensions 1.5 (Olympus) and ImageJ (Fiji).

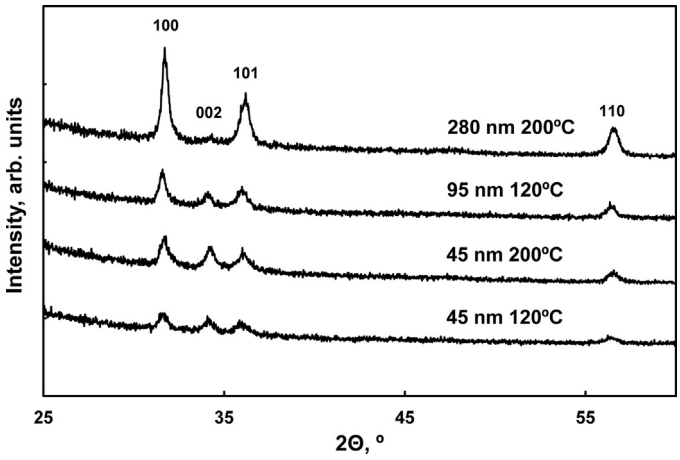

Fig. 1. X-ray diffraction patterns using a glancing angle for zinc oxide with various thicknesses deposited at 120 °C and 200 °C.

3. Results and discussion

ZnO thin films with various thicknesses were deposited by ALD at reaction temperatures of 120 °C and 200 °C. Fig. 1 shows the X-ray diffractograms of the ZnO films.

Using a glancing angle of 0.2°, the crystal structure of ZnO was found to be the hexagonal, wurtzite phase. The ZnO films were polycrystalline and the dominating peak was (1 0 0) but also (0 0 2), (1 0 1), and (1 1 0) orientations were found in all the films. The XRD patterns were also acquired with Bragg-Brentano geometry and the results showed the same trend with (1 0 0) being the strongest peak (not presented here). In contrast to these results studies have shown that (0 0 2) is the thermodynamically most stable surface (0 0 2) and it has been the most common in the ALD grown ZnO films over a range of temperatures (130–300 °C) and substrates (glass, Si (0 0 1), sapphire (0 0 0 1)) [7,37–40]. The evolution of the peak height suggests that the crystals are randomly oriented in the initial growth stages but as film thickness increases there are few