

# Influence of Zinc oxide films structure on biological protein adsorption for SAW biosensors

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## Abstract:

Zinc oxide films used for ZnO/SiO<sub>2</sub>/Si based surface acoustic wave (SAW) Love mode sensors, were fabricated. ZnO films surface structure will affect the adsorption of the biological protein on the surface, which has an influence on the sensitivity of the SAW sensors. The structural properties of ZnO films prepared by RF magnetron sputtering have been characterized by Thornton's structure zone model. The X-ray diffraction (XRD) patterns show that the films are highly oriented with their crystallographic c-axis perpendicular to the substrate. Bovine serum albumin (BSA) was fixed to the surface of ZnO films. Then the mass of the adsorption proteins was examined by AFM to find out the optimum ZnO films structure for biological protein adsorption.

**Key words:** ZnO films structure; BSA; Protein adsorption;

## Introduction

Zinc oxide (ZnO) is a wide band gap ( $E_g=3.37\text{eV}$ ) semiconductor with important piezoelectric and electronic properties. Due to its large specific surface area, good biocompatibility, chemical stability, biomimetic and high electron mobility features, Nanostructured ZnO has attracted much attention in biosensors research field. In addition, the ZnO with a high isoelectric point ( $\text{IEP}=9.5$ ) is suitable for the adsorption of low IEP proteins, and is a very promising material for applications in biosensors based on the mass loading [1,2,3]. A kind of SAW biosensors based on ZnO/SiO<sub>2</sub>/Si was reported by Soumya Krishnamoorthy and Thaleia Bei [4, 6]. But the influence of Zinc oxide films structure on biological protein is not clear.

In this paper, ZnO thin films with different structural properties were deposited on SiO<sub>2</sub> (50nm)/Si (100) substrates by changing Ar pressure according to Thornton's structure zone model [3] by RF magnetron sputtering, which is possible to obtain good orientation and uniform films close to single-crystal morphology even on amorphous substrate or at low substrate temperature [6,7,8]. XRD and SEM were employed to observe the surface microstructure of films, Bovine serum albumin (BSA), which

was widely used as a bridge to other proteins in biochemical tests, was chosen as a sample to study the Influence of Zinc oxide films structure on biological protein adsorption. BSA was fixed to the surface of ZnO thin films, and the adsorption of BSA was checked by AFM. In Further research, ZnO films would be used as piezoelectric film in ZnO/SiO<sub>2</sub>/Si SAW biosensors.

## Experiments

### 1. ZnO films fabrication

ZnO films were deposited on SiO<sub>2</sub> (50nm)/Si (100) substrates by RF magnetron sputtering system using a ZnO target (99.9%). The substrates were thoroughly cleaned with deionized water, ethanol and dried before loading in the sputtering system. The distance between the target and the substrate was 70 mm. The chamber was pumped down to  $5 \times 10^{-4}$  Pa by a turbomolecular pump. The substrate and the chamber wall were grounded and the substrate holder was heated from room temperature to 250°C before deposition. Throughout all experiments, the target was pre-sputtered for 10 mins under 100W RF power before the deposition to delete any contamination on the target surface. The films were grown at 250°C, power of 100 W and Ar pressure of 0.2-3.2 Pa for studying the changes

of the structural properties of ZnO thin films, which have been characterized in Thornton's structure zone model (Fig.1). The ZnO films were annealed at 900°C for 1 h. The heating rate is 150 °C/h from room temperature to annealing temperature. The crystallographic structure of the ZnO films was analyzed by XRD and SEM.

## 2. Surface activation and immobilization process

The ZnO films were activated by hydroxylation and followed by silanization with 3-aminopropyltriethoxysilane (ATES), used as the bridge to which glutaraldehyde is bound. Then BSA was immobilized on the surface of the ZnO films, which is used as a bridge to bind different proteins, and the mass of BSA were examined.

The ZnO films were washed several times with ethanol and deionized water. Then, the samples were immersed in ATES solution for 4h at room temperature. The silanized devices were washed many times with ethanol and deionized water and baked at 110°C. The silanized samples were then immersed in a 2% glutaraldehyde solution in 10 mM sodium phosphate-buffered saline (PBS) at pH 7.4, and shaken for 12 h at 4°C. The samples were washed many times with deionized water. The samples were immersed in BSA in PBS solution and incubated shaking for 12–18 h at 4°C, then rinsed several times in 10 mM of PBS, rinsed in ultra pure water, and dried in N<sub>2</sub> gas.

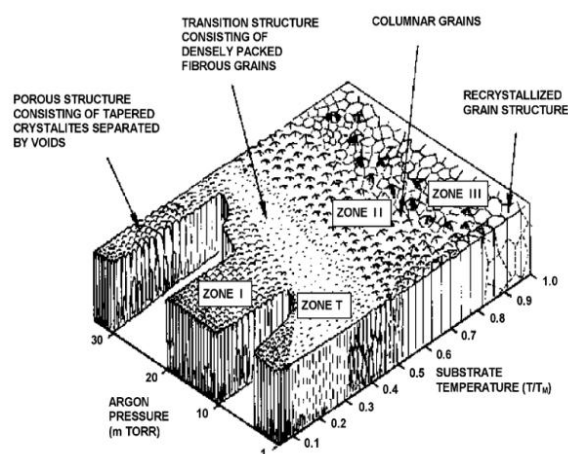


Fig. 1 Thornton's structure zone model for sputtered films on substrate temperature and argon pressure.

## Results and discussion

### 1. Crystallization quality and film microstructure

XRD was employed to characterize the nano-ZnO films. Fig. 2 shows the X-ray  $\theta$ -2 $\theta$  diffractogram of ZnO film deposited on SiO<sub>2</sub>/Si substrate with Ar pressures 1.2 Pa. We

observed that all of ZnO films have a strong diffraction peak of (002) at  $2\theta$  value of 36.48, independent of Ar pressure. The presence of a strong diffraction peak of (002) indicates that films have a (002) preferred orientation (c - axis).

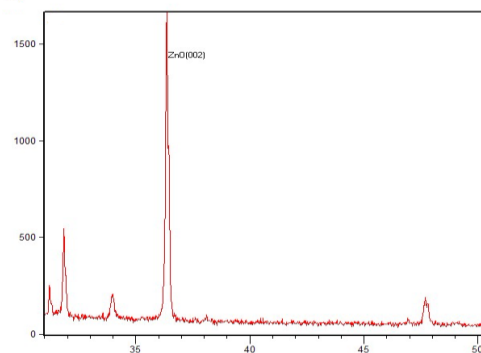
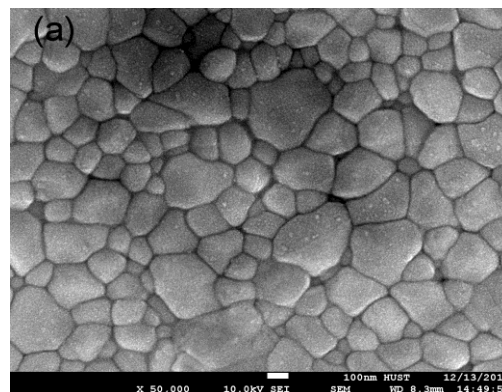


Fig. 2 XRD spectra of ZnO film deposited on SiO<sub>2</sub>/Si at 250 °C, RF power 100 W and Ar pressure of 1.2 Pa

The scanning electron microscopy (SEM) photographs of these films have been taken to evaluate their surface morphology and microstructure. As shown in Fig. 3, film's microstructure is in agreement with the trend given by Thornton's structure zone model, which was proposed by Movchan and Demchishin and extended by Thornton in Fig. 1. In the structure zone model, the surface structure was classified in term of four zones as a function of T/T<sub>m</sub> and argon pressure, where both T and T<sub>m</sub> are substrate temperature and coating material melting point, respectively. The T<sub>m</sub> of ZnO is 2250 K, and the value of T/T<sub>m</sub> is 0.23 at substrate temperature of 250 °C.

We found the surface structures of ZnO films prepared by different Ar pressure are different. The grain size of ZnO decreased with Ar pressure increased, and the surface of ZnO films became more-rough as Ar pressure increased. This is because the energy of particles arriving at the surface decreases with increasing argon pressure, leaving less energy for surface diffuse due to collisions with more atoms [9,10].



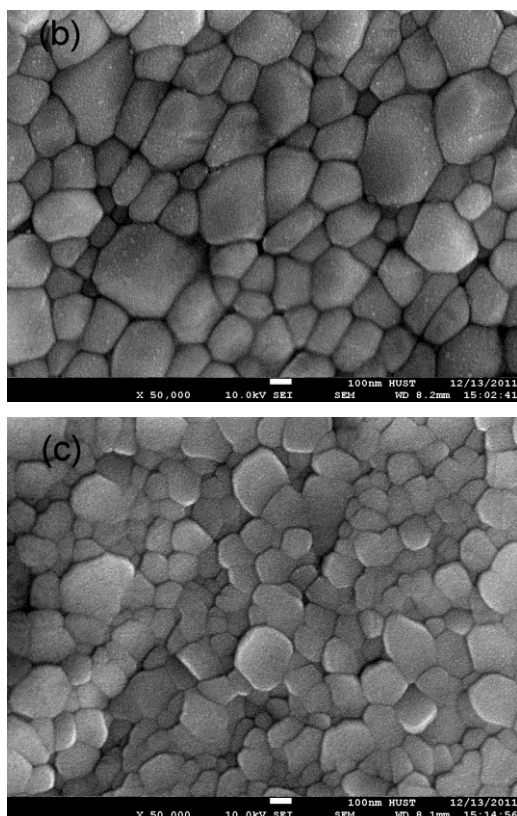


Fig. 3 The SEM micrographs of ZnO films deposited on SiO<sub>2</sub>/Si at 250 °C, RF power 100 W and Ar pressure of (a)  $P_{Ar}=0.2$  Pa; (b)  $P_{Ar}=1.2$  Pa and (c)  $P_{Ar}=3.2$  Pa

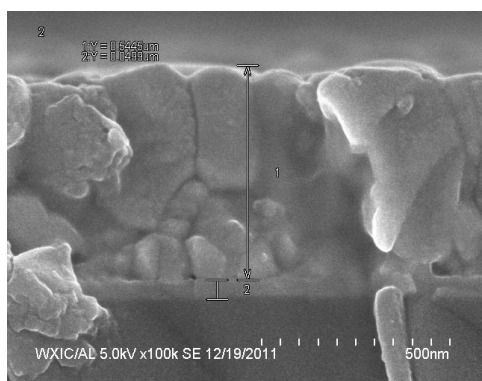


Fig. 4 Sectional view of the sample deposited for 50min and annealed at 900 °C

The thickness of ZnO film and that of SiO<sub>2</sub> are shown in Fig. 4. The thickness of ZnO film is 544.5 nm while that of SiO<sub>2</sub> is 50nm. The surface of the films is smooth and the grain of crystal is compact.

## 2. Protein adsorption analysis

Due to a high concentration of unsaturated bonds on ZnO films surface, ZnO films are suitable for the adsorption of proteins. In this paper, we fixed BSA to the activated ZnO films surface. AFM photographs of these samples have been taken to evaluate adsorption of BSA on ZnO films.

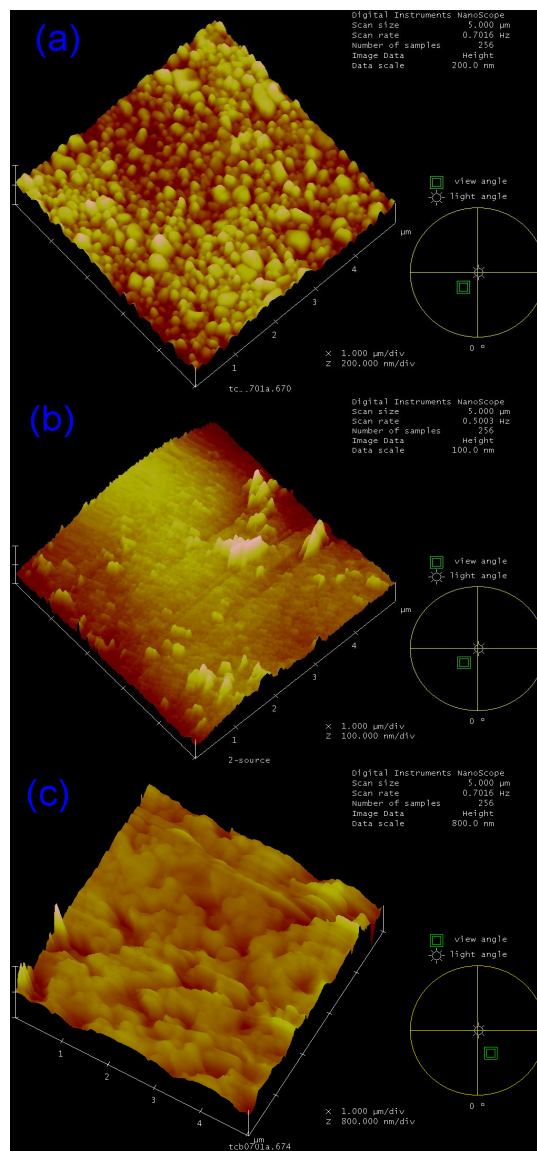


Fig. 5 AFM images of ZnO film and BSA (a) AFM image of ZnO film prepared at Ar pressure of 1.2 Pa (b) AFM image of BSA bound onto ZnO film prepared at Ar pressure of 0.2 Pa (c) AFM image of BSA bound onto ZnO film prepared at Ar pressure of 1.2 Pa

Fig. 5 is AFM images of the area of 5  $\mu\text{m} \times 5 \mu\text{m}$  window on ZnO films. As shown in Fig. 5 (a), the grain size of ZnO prepared at Ar pressure of 1.2 Pa is about 100nm-200nm, in accord with the grain size SEM observed before. Fig. 5 (b) is AFM image of BSA bound onto ZnO film prepared at Ar pressure of 0.2 Pa. The surface became smooth, that is to say, BSA was fixed to surface of ZnO films. Fig. 5 (c) is AFM image of BSA bound onto ZnO film prepared at Ar pressure of 1.2 Pa. As shown in Fig. 5 (c), the loose porous structure of BSA is in accord with the structure of protein and illustrated that more BSA were adsorbed on ZnO films prepared at Ar pressure of 1.2 Pa than that on ZnO films prepared at Ar pressure

of 0.2 Pa. The thickness of BSA in Fig.5 (c) was also larger than that in Fig.5 (b). That is to say, the protein adsorption increased with grain size of ZnO decreased, and ZnO films with columnar crystalline structure adsorbed more proteins (Fig. 5).

As we explained above, the energy of particles arriving at the surface decreases with increasing argon pressure, leaving less energy for surface diffuse due to collisions with more atoms. In addition, adsorbed argon limits also the mobility of surface's atoms and self-shadowing effect becomes pronounced at high argon pressure. So the protein adsorption increased with Ar pressure increased, while the grain size of ZnO decreased with Ar pressure increased.

### Conclusion

ZnO thin films deposited on SiO<sub>2</sub>(50nm)/Si (100) substrates made by RF magnetron sputtering at substrate temperature of 250 °C and RF power of 100 W have been studied by change of Ar pressure. The influence of Ar gas pressure on characteristics of sputtered ZnO films was examined. The X-ray diffraction reveals that the films are oriented with their c-axis perpendicular to the substrate surface, almost independent on the Ar pressure. The grain size of ZnO decreased with Ar pressure increased, and the surface of ZnO films became rougher as Ar pressure increased. ZnO crystalline grain became columnar crystalline structure as Ar pressure increased. The protein adsorption decreased with grain size of ZnO decreased, and ZnO films with columnar crystalline structure adsorbed more proteins.

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