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# Nanostructured ZnO films for anti-adhesion activity against *S.aureus*

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## ABSTRACT

Emergence of bacterial adhesion causes severe infections in implants which lead to device failure. Adhesion of bacteria can be diminished by adapting the surface of the material. ZnO films were grown by reactive dc magnetron sputtering onto thoroughly cleaned stainless steel and glass substrates with diverging the cathode power from 20 to 40 W. The sputtered films were characterized using X-ray diffraction (XRD), Field emission scanning electron microscopy (FE-SEM) and UV-Visible Spectroscopy to study the structural, morphological and optical properties. XRD results proved that the sputtered films were crystalline in nature with preferred (002) orientation and the crystallite size were increased. FE-SEM shows the films formed with spherical morphology. Upon increasing the sputtering power the transparency of the films increased from 75 to 99 % and the thickness of the films was increased from 171 to 187 nm. The ZnO films deposited at 30 W cathode power exhibited better anti adhesion activity against *Staphylococcus aureus* (*S.aureus*).

## KEYWORDS

Thin film; ZnO; Sputtering; *S.aureus*; Adhesion.



## INTRODUCTION

Biofilm formation is the major cause for implant failure and many researchers focusing on this to overcome this problem since the number of internal fixation devices forecast to increase 7.7 % annually in 2015[1]. Infections rates are higher after in the revision of surgery than the primary replacement. Biofilm hinders immune activity and decreases phagocytosis, complement activation, opsonization etc.[2] US Health care shown the medication expense of biofilm related infections is around three billion dollars[3,4]. Biofilm growth is massive and very difficult to control and it is enriched with persister cells. Surface modification using thin films to introduce antibacterial activity is a strategy to prevent biofilm adhesion and to improve the life time of implants. Among various materials, ZnO is one of the most attractive materials since it is inorganic metal oxide, inexpensive material, biocompatible, hemocompatible and has antibacterial activity. Inorganic antibacterial executors are safer and have better stability than organic bacterial agents[5]. Because of the excellent properties of ZnO, it might be utilized as a wide range of applications, for example, biosensor[6,7], gas sensor[8,9], UV sensor devices[10], inverted polymer solar cells[11], waste water treatment[12], etc.

In the present work, ZnO thin films were deposited by reactive dc magnetron sputtering on stainless steel (AISI SS 304) and glass substrates by varying the cathode power from 20 to 40 W and their structural, morphological and optical properties were studied. And also the biofilm activity of the films was studied towards *S.aureus*.

## EXPERIMENTAL PROCEDURE

ZnO films were deposited by reactive dc magnetron sputtering on to thoroughly cleaned stainless steel substrates [9]. Initially stainless steel substrates were soaked in 10 % sodium hydroxide solution and then ultrasonically cleaned using acetone and ethanol for 10 min in each solution and then dried in hot air oven for 1 h[13,14]. The cleaned substrates were loaded on to the substrate holder. The zinc target (99.99 % purity) was sputter cleaned for 15 minutes in argon (15 sccm) to discard the contaminants on the target surface. After the sputter cleaning, oxygen gas (5 sccm) was allowed inside the deposition chamber and the working pressure during deposition was maintained as $5.1 \times 10^{-3}$ mbar. The substrate biasing was maintained as -100 V. The source to substrate distance was fixed at 3 cm and the deposition time was 15 minutes. The films were deposited at three different cathode powers, viz., 20, 30, and 40 W, which yielded the films of thicknesses 180, 230 and 275 nm (± 5 nm) respectively as measured by stylus probe profilometer (Mitutoyo, SJ 301, USA).

The structural properties of the films were investigated by XRD (Rigaku Ultima III, Japan) technique using $CuK_{\alpha}$ radiation. The surface morphology of the films was analyzed using FE-SEM (JEOL JSM - 6701 F, Japan) with 3 kV electron beam power and at a working distance of ~ 7 mm. The optical properties of the films were studied by using UV-Vis spectroscopy (Perkin Elmer, USA) in the wavelength range of 200 - 800 nm.

The ZnO films were placed in 24 – well plate. The plasma proteins was diluted to 50 % using carbonate buffer (1:2 ratio of Sodium Carbonate and Sodium bicarbonate) solution and 500 µl of diluted plasma proteins was poured into each well of the plate. The plate was incubated at 37 °C for 24 h to form a layer of plasma over the samples. The blood plasma was removed from each well and 100 µl of prepared *S.aureus* culture was poured into each well. Following this 1ml of Biofilm medium which containing Tryptic soy broth (TSB) supplemented with 3.0 % NaCl (Wt/Vol) and 0.5 % dextrose was poured into each well and the plate was kept in the incubator at 37 °C for 7 days. The biofilm medium was replaced in its entirety at 24 hour intervals for 7 days to allow bacterial adhesion[15].

After seven days, biofilm medium were removed from wells and the samples were washed thrice with phosphate buffered saline (PBS) solution to remove the non-adherent bacterial cells. Adherent bacterial cells present on each sample were removed using properly sterilized cell scraper. The cells from each sample were serially diluted to form bacterial suspension using distilled water. Biofilm medium along with agar was prepared; poured in to petriplates and allowed it to solidify. Bacterial

suspensions were inoculated in to agar plates. In these tests, the plating out of each dilution was done in triplicate. The culture plates were then incubated at 37 °C for 24 h and bacterial cell counts were calculated.

## RESULTS AND DISCUSSION

### Structural studies

Figure 1 shows the XRD patterns of ZnO films deposited over stainless steel substrate at different powers from 20 to 40 W. It was confirmed that all the deposited films exhibited hexagonal wurtzite structure with preferred c-axis orientation in (002) plane. At 20 W, minimum intensity of the peak was obtained due to the lack of kinetic energy of the ZnO atoms. The crystallinity of the film was enhanced with increasing power due to increase in number of ions and electrons in the plasma and the sputtered atoms gained high energy from positive ions because of collision.

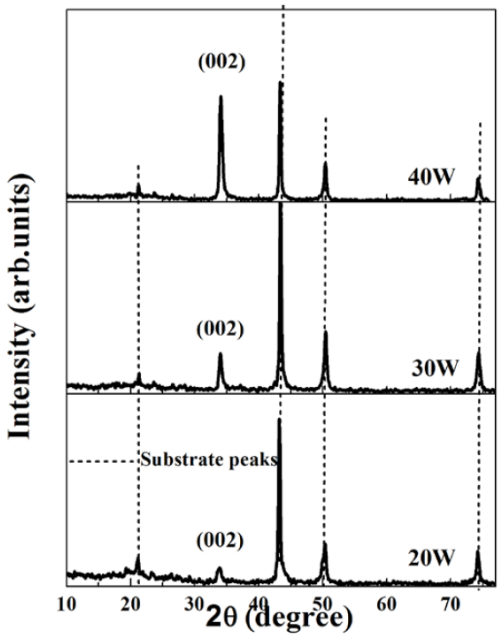


**Figure 1: XRD patterns of ZnO films deposited at different cathode powers**

The grain size of the films was calculated using Scherrer's formula

$$D = 0.9\lambda/\beta\cos\theta$$

where, λ is the wavelength (Å), β is the full width half maxima (radians) and θ is the diffraction angle (radians).

On increasing the sputtering power from 20 to 40 W, the grain size value of the film was increased from 17.6 to 21.4 nm and the grain boundaries were reduced and this leads to the growth of defect free films.

### Morphological studies

The FE-SEM micrographs of ZnO films deposited at different powers are shown in Figure 2. It was confirmed that the growth of the films depends on the cathode power. The growth and spherical morpholgy of the films was increased with increasing the power. In 20 W, the film was in nucleation state and it does not form uniform film. The ZnO films grown at 30 and 40 W exhibited better growth and conitinuous and uniform surface with increased grain size as observed in XRD.

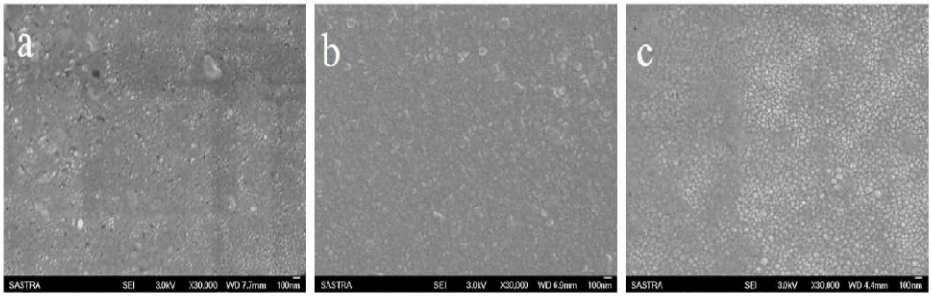


**Figure 2: FE-SEM micrographs of ZnO films deposited at different cathode powers of a) 20, b) 30 and c) 40 W**

## Optical properties

The transmittance spectra of the deposited ZnO films are shown in Figure 3. The transparency of the films was increased on increasing the power until 30 W because of the removal of crystalline defects at higher sputtering power. At 40 W, transparency was decreased due to scattering effect as shown in Figure 3.

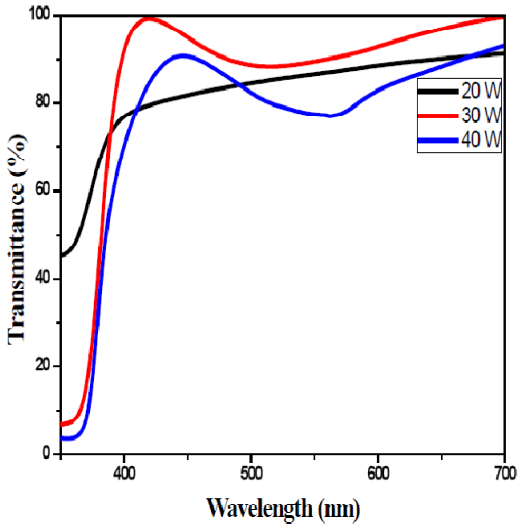


**Figure 3: Optical transmittance of ZnO films deposited at different cathode powers**

## Bacterial adherence studies

The bacterial adhesion studies were analyzed using clinically isolated *S.aureus* and colony forming units were calculated. Large number of colonies was formed over the untreated stainless steel substrate and substantial amount of colonies were reduced over the deposited films. At 20 W, minimal amount of colony formation was reduced because films were in nucleation state and the uniform films were not formed. Anti-adhesion properties were improved with increasing the power due to the continuous film formation. The number of colony forming units (CFUs/ml) with different powers was shown in Figure 4. Maximum number of colonies was inhibited at 30 W due to the continuous film decreases the release of iron ions which provides the essential environment for bacterial adhesion.

The percentage of bacterial adhesion was calculated using logarithmic values of CFUs and this shows all the deposited films shows the inhibition of *S.aureus* shown in Figure 5. Among these 68 % of bacterial adhesion was found over the films deposited at -100 V. This may be due to the release of $Zn^{+}$ ions, disrupts the outer membrane of bacteria and may induce the production of reactive oxygen species or due to the electrostatic interaction between cell surfaces and ZnO and damages the cell wall of bacteria leads to cell lysis[16].

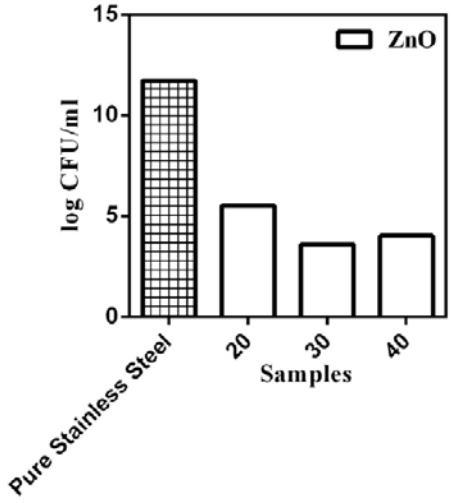


**Figure 4: Colony forming units over the samples**

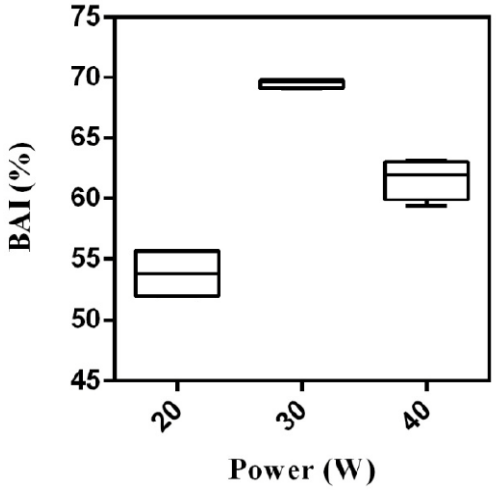


**Figure 5: Percentage of bacterial adhesion inhibition over the films**

## CONCLUSION

ZnO films were sputter deposited on AISI SS 304 substrates by reactive dc magnetron sputtering at different cathode powers and their structural, morphological, optical properties and antibacterial activity were studied. Among the various sputtering powers used in the present study, the ZnO films deposited with 30 W cathode power exhibited maximum anti-adhesion activity against *S.aureus*.

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