Antibacterial and Hemocompatibility of SnO/ZnO Coated Titanium for Implant Applications

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**Abstract :**In the biomedical field, the primary objective is to develop versatile biomaterials which are capable of action against bacterial growth. Among the various challenges, chronic infections stand out as the most severe and destructive complications associated with biomaterial usage. This issue is of utmost significance in the field of orthopedics and dental implants. In this present study, we develop a biocompatible and antibacterial coating that was fabricated by electrophoretic deposition (EPD).The coating formation was investigated by surface characterization studies including surface morphology and functional group analysis which confirmed SnO/ZnO on the Cp-Ti surface. The surface exhibited a laminar array with petal-like morphology which enhances the osteointegration process. *In-vitro* analysis of hemocompatibility studies exhibited the coating as a hemocompatible material. Antibacterial studies showed that coating acter against *S.aureus* and *E.coli* bacteria.

**keywords:** Biomaterials, good load-bearing application, titanium-based materials, electrophoretic deposition is a mutual colloidal dispensation

# Introduction

Biomaterials are typically described as materials that have been purposefully designed to control and influence the progression of diagnostic or therapeutic procedures through the regulation of their interactions with elements within living systems [(Williams, 2009)](https://paperpile.com/c/ZYuR9B/71LB). Biomaterials are classified into different types such as metals, ceramics, polymers, and synthetic and natural materials are used to recreate the structure of bone and parts of the human body[(Dharman et al., 2021)](https://paperpile.com/c/ZYuR9B/YIy9a). Furthermore, metallic biomaterials are used for orthopedic and dental implants because they have superior resistance and load-bearing materials [(Hanawa, 2018)](https://paperpile.com/c/ZYuR9B/MKq3). Currently, titanium, Co-Cr alloys and 316L Stainless Steel are used in orthopedic applications. Among these metallic materials, Titanium and its alloys have high corrosion resistance, good load-bearing application and excellent mechanical properties along with biocompatibility [(Walters, 2019)](https://paperpile.com/c/ZYuR9B/67sV).

In addition to their familiar application as hip and knee prostheses, titanium-based materials are also utilized for trauma plates, dental implants, and bone screws [(Neha et al., 2021)](https://paperpile.com/c/ZYuR9B/F0Dch)[(Maliael et al., 2021)](https://paperpile.com/c/ZYuR9B/w5Qf3)[(Lakshmi, 2021)](https://paperpile.com/c/ZYuR9B/tFGSS). It has been reported that over 1,000 tonnes of titanium-based materials are implanted in the human body worldwide annually, and this quantity is projected to steadily rise over the next decade [((Sam) Froes, 2018)](https://paperpile.com/c/ZYuR9B/XmGT). Ti has been traditionally modified with surface engineering treatment to enhance the properties including modifying the surface treatment methods. Surface treatment include anodization, electrophoretic deposition, chemical vapor deposition, physical vapor deposition, etc. [(Jiang et al., 2023)](https://paperpile.com/c/ZYuR9B/HJQH).Among various methods, electrophoretic deposition is a mutual colloidal dispensation method often employed in ceramic coating [(Obregón et al., 2019)](https://paperpile.com/c/ZYuR9B/dFJX). It offers numerous advantages, including rapid formation process and minimal constraints on substrate shape. Initially, charged particles are dispersed or suspended within a liquid medium and subsequently directed towards an electrode of opposite charge through the application of an electric field, leading to the creation of a deposited film [(Pikalova & Kalinina, 2019)](https://paperpile.com/c/ZYuR9B/dVxF). For example, Hoomehr et al. produced composite core-shell nanoparticles of bioactive glass-zirconia and effectively applied them onto a Ti6Al4V substrate using a single-step EPD process [(Hoomehr et al., 2021)](https://paperpile.com/c/ZYuR9B/n7dF). An alternative method of metal oxide coating has enhanced antibacterial activity with the composite coating[(Sabarathinam & Madhulaxmi, 2021)](https://paperpile.com/c/ZYuR9B/1Ud5d)[(Sushanthi et al., 2021)](https://paperpile.com/c/ZYuR9B/09zD4)[(Harsha et al., 2022)](https://paperpile.com/c/ZYuR9B/NGaJU). There are reports of Sn coated on Ti metal. Interestingly Tibayan et al, have studied that tin oxide nanoparticles displayed antibacterial characteristics. They evaluated the effectiveness of Ag/SnO2 nanocomposites as coating materials with strong antibacterial capabilities [(Tibayan et al., 2020)](https://paperpile.com/c/ZYuR9B/9eHA). In certain situations, antimicrobial coatings are required to maintain high transparency within the visible spectrum for various applications [(Tiwari & Jain, 2023)](https://paperpile.com/c/ZYuR9B/DASZa)[(Graf et al., 2023)](https://paperpile.com/c/ZYuR9B/HowE4). Various transparent antimicrobial coatings, such as those based on zinc oxide (ZnO, ZnO:Co, ZnO:Sn ZnO-Y2O3 ZnO-CeO2 ), have been synthesized and examined [(Evstropiev et al., 2019)](https://paperpile.com/c/ZYuR9B/yZ8A). The main reason for long-term implant failure isbacterial infection associated, during osteointegration process. To overcome this drawback, the present work demonstrates that the Electrophoretic deposition (EPD) of SnO/ZnO composite coating on titanium enhances the corrosion resistance and antibacterial activity.

# Materials and Methods

## Preparation of Coating

The medical grade of commercially pure titanium (Cp-Ti) was purchased from Ti Anode Chennai, India(Rafi et al., 2024). The sample dimension was 1.5 cm x 1.5 cm x 2 mm thickness and the sample was grounded in SiC paper from 250 to 1500 grade to remove debris and to make an even surface. After polishing the sample was kept with acetone and double distilled water using ultrasonication to clean the surface. Then the sample was treated with Kroll’s reagent for 10 seconds (Tuluwengjiang et al., 2024). They were washed with DD water and kept with a desiccator.

The tin chloride (SnCl2.2H2O) was purchased from (Merck 98.5% purity) and the Zinc acetate dihydrate (CH3COO)2Zn.2H2O) was purchased from Sigma Aldrich (98 % purity). The 0.1 M of NaOH was prepared for 50 ml DD water and 0.3 g of (CTAB) was added to the solution. Then 0.5 g of tin chloride was added with a mass ratio of 1:1 of zinc acetate solution after the solution was stirred in 1 h. The solution was transferred to an autoclave for the hydrothermal process at 10 h at 90º C. Then the solution was examined by the EPD process. In the EPD process, the potential was applied at 20 V for 10 min, where platinum was the cathode and Ti was the anode to deposited coating. Further, the sample was used in characterization studies

## Surface Characterization studies

The coated sample was examined by the surface topography analysis using Field Emission-Scanning Electron Microscopy with Energy Dispersive X-ray analysis (FE-SEM/EDX) and the JEOL model (JSM-IT800 NANO SEM). The presence of the functional group at the coated sample was confirmed by the FT-IR spectrum using an Alpha II Bruker model spectrometer from the wave number of 4000-400 cm-1 range.

## Hemocompatibility

According to the established procedure outlined by [(Samsonenko et al., 2019)](https://paperpile.com/c/ZYuR9B/qzRV), a hemolysis test was conducted. To do this, trisodium citrate (3.2%) was mixed with human blood collected from donors in the correct 9:1 ratio. The samples were then incubated with phosphate buffer saline (PBS) for 30 minutes at 37 °C. Subsequently, anti-coagulated blood was loaded into standard tubes and incubated at 37 °C. After incubation, the tubes were centrifuged at 3000 rpm for 10 minutes, using a positive control of 0.1% sodium carbonate and anti-coagulated blood, as well as a negative control with PBS. The following calculations were performed to determine the hemolysis rate.

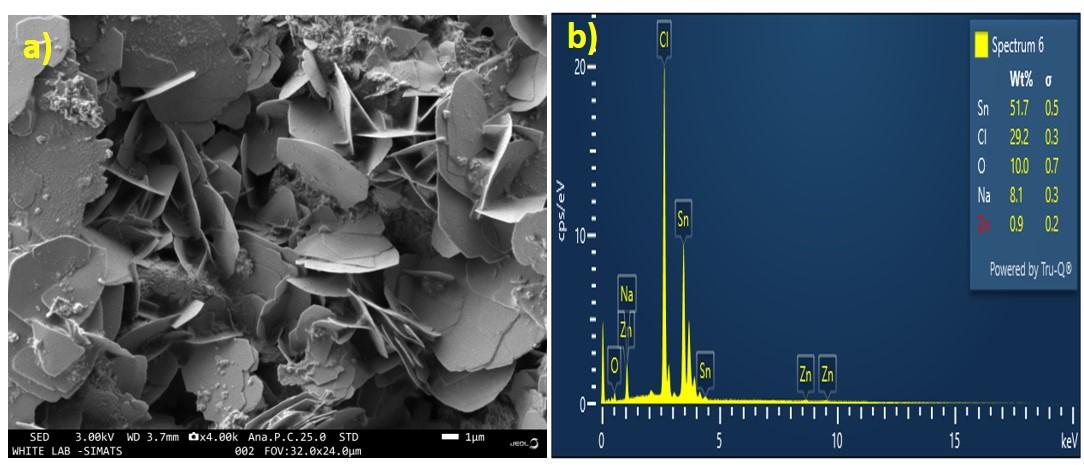
## Antibacterial activity

Two types of bacteria namely the Gram-negative strain *E. coli* and the Gram-positive strain *S. aureus* were investigated in this study. These bacterial cultures were retrieved from frozen stock and then transferred to Trypcase Soy Agar (TSA) plates. The plates were subsequently placed in an incubator at 37 °C for a duration of 18–24 hours. Following this incubation period, the bacteria were transferred to 50 mL of sterile Tryptic Soy Broth (TSB) medium and allowed to grow for 18–24 hours at 37 °C with agitation at 80 rpm. Before inoculation, the bacterial strains were subcultured into fresh TSB at a 1:50 ratio and incubated for 2 hours at 37 °C with agitation at 80 rpm.

# Results

## Surface Morphology analysis

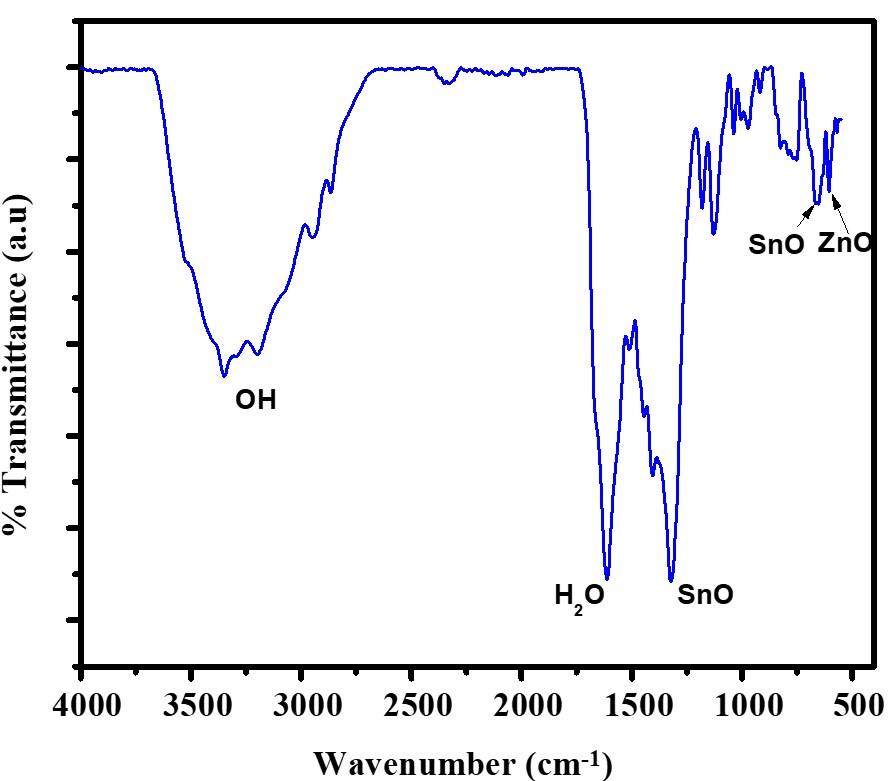
The surface morphology and elemental analysis of the coated sample are given in Fig.1. The surface exhibited a laminar array with petal-like morphology on the coated SnO/ZnO on Cp-Ti surface. The elemental dispersive analysis confirmed that the Sn, Zn, O, Cl and Na revealed the EDX profile. The surface are interlinked with each other and self-assembly porous structure was formed and the coating shows good adhesion on Cp-Ti surface. Surface morphology plays a role in the osteointegration process. The FE-SEM revealed porous morphology under high magnification, which aid in the osteointegration process.



**Figure 1**. EPD coating of a) SEM and b) EDX profile of Sno/ZnO coated Ti

# ATR-IR studies

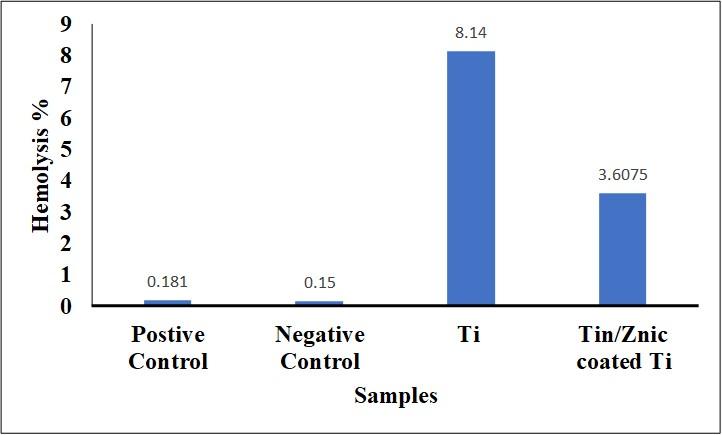
The functional group analysis of the coated sample was examined by FT-IR spectra (Fig.2). The stretching vibration of the hydroxyl group (OH) and water molecules appeared at the broadband in the wavenumber of 3000-3500 cm-1 and 1610 cm-1 respectively. The peak ascribed around 600 to 500 cm-1 stretching vibration of SnO and an O-Sn-O bending vibration band appeared [(Samsonenko et al., 2019)](https://paperpile.com/c/ZYuR9B/qzRV). The nanoparticles of the SnO were formed at the peak of 1325 cm-1 which was in agreement with SEM images that form the nanostructure [(Suresh et al., 2020)](https://paperpile.com/c/ZYuR9B/gWc0). A small intensity peak was observed at the ZnO indicating the 597 cm-1 [(Alamdari et al., 2020)](https://paperpile.com/c/ZYuR9B/ZXPq). The base metal oxide of the (Ti-O) peak overlapped with the ZnO. This spectrum was confirmed the SnO/ZnO-coated Cp-Ti metal.



**Figure 2.** FT-IR spectra of SnO/ZnO coated Cp-Ti

## Hemolysis

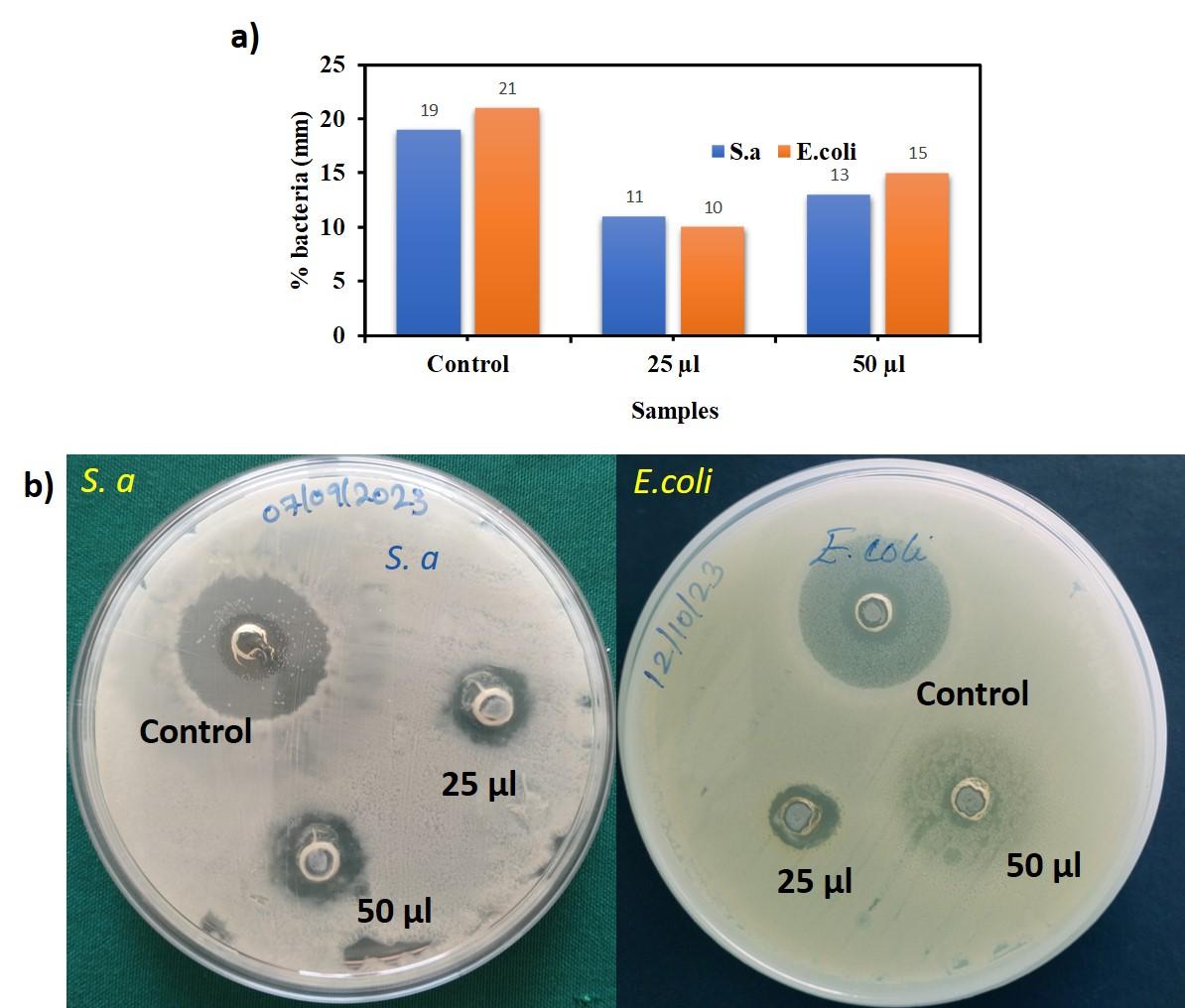
The detrimental impact of biomaterial toxicity directly influences Red Blood Cells (RBCs), resulting in hemolysis. When the bone implant material is initially implanted, it interfaces with the blood tissues during the coagulation process. In our study, the hemolysis of the uncoated (Ti) sample and SnO/ZnO coated Ti sample was evaluatedare (Fig. 3). Further evaluation of the uncoated (Ti) sample exhibited the hemolytic nature of the 7.8 % lysis rate. EPD coating of SnO/ZnO on the Ti surface revealed only hemolytic nature 3.8 % lysis rate. According to the ASTM standard hemocompatibility < 2% is non-hemolytic, < 2-5 % is slightly hemolytic and above 5% is hemolytic nature [(Kalaiyarasan et al., 2023)](https://paperpile.com/c/ZYuR9B/M8UP). In our study, the material showed slightly hemolytic and thus confirming it as biocompatible material which can be used for implant applications.



**Figure. 3**. Hemocompatibility of uncoated and coated Ti samples

## Anti-bacterial activity

The antibacterial activity of the coated sample was evaluated by the Zone of inhibition (ZOI) method. Initially, the samples were immersed in PBS solution for 24 h incubation, the extract was used as different concentrations were evaluated and the control used as an antibiotic Fig.4. The zone of inhibition percentage observed at 25 µl exhibited the *S. aureus* and *E.coli* exhibits 11 and 10 % respectively. The 50 µl concentration revealed that 13 and 15 % of the zone. The bacteriostatic force of SnO/ZnO particles killed the cell membrane of bacteria resulting in cell death [(Murali et al., 2021)](https://paperpile.com/c/ZYuR9B/joaH).



**Figure 4.** a) Percentage of the zone and b) Zone of the inhibition plate method of *S.aureus* and *E.coli* of coated Ti sample

# Discussion

The antibacterial properties of the coating on Cp-Ti are very important for clinical applications[(Jabin et al., 2021)](https://paperpile.com/c/ZYuR9B/QJyxU)[(Balaji Ganesh S & Sugumar, 2021)](https://paperpile.com/c/ZYuR9B/ByEXS) [(Govindaraj & Dinesh, 2021)](https://paperpile.com/c/ZYuR9B/U2GXq). Nowadays,titanium is used the most common implant for orthopedic and dental implants. These implants may fail due to the antibacterial and biofilm formation of the initial stage, before the osteointegration process [(Ajay, Suma, et al., 2022)](https://paperpile.com/c/ZYuR9B/zxikR) [(Katyal et al., 2021)](https://paperpile.com/c/ZYuR9B/7Rks4). In our study coating, the electrophoretic deposition of the Ti sample was coated on SnO/ZnO by applying a potential of 20 V for 10 min. A Bloniarz et al, have fabricated SnO/Sr with bioglass on Ti substrate to enhance the antibacterial activity and bioactivity of the coating [(Błoniarz et al., 2022)](https://paperpile.com/c/ZYuR9B/TRWd).The β type of Ti alloy has demonstrated TiO2 nanotubes for electrochemical processes and incorporation of Sn reduces the bacterial attachment used in orthopedic applications [(Verissimo et al., 2015)](https://paperpile.com/c/ZYuR9B/yViF). In their study, Henry et al. investigated the antibacterial properties of SnO2 thin films were applied to a glass substrate using the sol-gel spin coating technique[(Ajay, Rakshagan, et al., 2022)](https://paperpile.com/c/ZYuR9B/jyM3G). They showed antibacterial property of thin SnO2 films against Escherichia coli and Bacillus, as assessed by the agar method. Bacterial growth was observed on the uncoated substrate, but no bacterial growth was detected on the SnO2-coated substrate[(Chidambaram et al., 2022)](https://paperpile.com/c/ZYuR9B/4IFAu).[(Ajay, Sasikala, et al., 2022)](https://paperpile.com/c/ZYuR9B/ZunAk). These indicate that SnO2 offers a promising solution for combatting bacteria through electrostatic interactions that disrupt bacterial membrane integrity and stimulate the production of harmful free radicals [(A.s. et al., 2023)](https://paperpile.com/c/ZYuR9B/SskY). The hemocompatibility of titanium and its alloy has outstanding properties required for blood contact implants caused by thrombosis and restenosis [(Harsha & Subramanian, 2022)](https://paperpile.com/c/ZYuR9B/Mehnw)[(Deepika et al., 2022)](https://paperpile.com/c/ZYuR9B/Miygv)[(Solanki et al., 2022)](https://paperpile.com/c/ZYuR9B/klLRq). The SnO/ZnO coating has been proved to be the blood-compatible coating [(Henry et al., 2015)](https://paperpile.com/c/ZYuR9B/BeoT). Georgeta et al, have studied the Co-doped ZnO enhanced the antibacterial and in-vitro bioactivity [(Manivasagam et al., 2021)](https://paperpile.com/c/ZYuR9B/6jXU). We observed also similar anti-bacterial activity of Ti-coated samples.

# Conclusion

In this study, electrophoretic deposition of SnO/ZnO coated on the Cp-Ti is a promising biocompatible coating which has proven to enhance the surface properties and antibacterial activity of the implants.

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