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*RESEARCH ARTICLE / ARAŞTIRMA MAKALESI*

# Electrodeposited ZnO Nanostructures on ITO Surfaces: Exploring Their Efficacy for Cholesterol Biosensing Applications

## ITO Yüzeylerinde Elektrodepozitlenen ZnO Nanoyapılar: Kolesterol Biyosensör Uygulamaları için Etkinliklerinin Araştırılması

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**Abstract**

In this study, ZnO nanostructures were prepared by electrochemical anodization of electrodeposited Zn on ITO/glass substrates for cholesterol detection. The efficiency of the developed ZnO nanostructures in the detection of the Cholesterol oxidase (ChOx) enzyme was determined by the cyclic voltammetry method. The XRD and SEM results confirmed the synthesis of ZnO nanostructures prepared by the anodization method with various parameters. The effect of electrodeposition and anodization time on the morphology was observed. Cyclic voltammetry of ZnO/Zn/ITO/glass and Pt/ZnO/Zn/ITO/glass electrodes in electrolytes with various cholesterol concentrations was performed. The detection limit of the obtained Pt/ZnO/Zn/ITO/glass structured electrode was calculated as $0.965x10^{-3}$M. The resulting material with a layered structure may have potential applications in electrochemical sensors and biosensors in biomedical applications. In addition to biosensing performance, this study proposes a new approach for the development of ZnO-based biosensors that does not require expensive infrastructure and raw material costs, making it possible to develop high-sensitivity biosensor electrodes with lower detection limits with improvements to be made in future studies.

***Keywords:*** *ZnO nanostructures, electrochemical anodization, cholesterol, biosensor*

**Öz**

Bu çalışmada, ZnO nanoyapıları, kolesterol tespiti için ITO/cam altlıklar üzerine elektrodepozitlenen Zn'nin elektrokimyasal anodizasyonu ile hazırlanmıştır. Geliştirilen ZnO nanoyapıların Kolesterol oksidaz (ChOx) enziminin tespitindeki etkinliği döngüsel voltametri yöntemi ile belirlenmiştir. XRD ve SEM sonuçları, çeşitli parametrelerle anodizasyon yöntemiyle hazırlanan ZnO nanoyapıların sentezini doğrulamıştır. Elektrodepozisyon ve anodizasyon süresinin morfoloji üzerindeki etkisi gözlemlenmiştir. ZnO/Zn/ITO/cam ve Pt/ZnO/Zn/ITO/cam elektrotların çeşitli kolesterol konsantrasyonlarına sahip elektrolitlerdeki döngüsel voltametrisi gerçekleştirilmiştir. Elde edilen Pt/ZnO/Zn/ITO/cam yapılı elektrotun tespit limiti $0,965x10^{-3}$M olarak hesaplanmıştır. Elde edilen katmanlı yapıya sahip malzeme, biyomedikal uygulamalarda elektrokimyasal sensörlerde ve biyosensörlerde potansiyel uygulamalara sahip olabilir. Bu çalışma, biyosensör performansına ek olarak, ZnO tabanlı biyosensörlerin geliştirilmesi için pahalı altyapı ve hammadde maliyeti gerektirmeyen yeni bir yaklaşım önermekte ve gelecek çalışmalarda yapılacak iyileştirmelerle daha düşük tespit limitlerine sahip yüksek hassasiyetli biyosensör elektrotlarının geliştirilmesini mümkün kılmaktadır.

***Anahtar Kelimeler:*** *ZnO nanoyapılar, elektrokimyasal anotlama, kolesterol, biyosensör*

## 1. Introduction

The utilization of biosensors in clinical diagnostics has recently garnered significant attention. Among the various clinical parameters, the measurement of cholesterol especially at higher levels holds particular importance due to its association with conditions such as cardiovascular diseases, obstructive, diabetes, nephrosis, jaundice, etc [1, 2]. The biosensor method, which often involves immobilizing an enzyme to an electrode, has become a critical way to estimate cholesterol. The selection of the immobilization technique significantly influences the performance of the biosensor [3]. Various techniques have been developed for the detection of cholesterol in blood plasma, including fluorometry, high-performance liquid chromatography, and electrochemical approaches.[4]. Among these methods, electrochemical biosensors are particularly noteworthy due to their high accuracy, low response time, and convenience. The use of modified electrodes in electrochemical assays for cholesterol analysis has significant attention from an increasing number of researchers [5, 6].

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For decades, researchers have been interested in material selection, design, structure, processing, morphology control, and optimization of electrochemical biosensors electrodes [7]. Metal oxide nanostructures have been widely used in the development of biosensor electrodes in recent years due to their thermodynamic stability, semiconductor properties, and cost-effectiveness [8–10]. Moreover, they can be produced by many methods to obtain the aforementioned properties expected from them [8]. ZnO has attracted a great deal of attention within the wide range of metal oxide materials. Notably, certain characteristics of ZnO, such as its high thermal and mechanical stability at room temperature and a binding energy of 60 meV, make it a versatile material for various applications [11]. Additionally, ZnO nanostructures exhibit remarkable attributes, including a wide band gap, non-toxicity, biocompatibility, chemical and photochemical stability, and excellent electron mobility features. These qualities render ZnO nanostructures suitable for the production of efficient biosensors [12, 13]. According to the review study of Beitollahi et al on ZnO nanostructures-based biosensors, the most approved production methods of ZnO nanostructures are reported as sol–gel method, double-jet precipitation, thermal evaporation, self-combustion, hydrothermal synthesis, solution synthesis, simple thermal sublimation, polymerized complex method, high energy ball milling, physical vapor deposition, chemical vapor deposition, etc [13–17].

On the other hand, in a recently published review, many different studies evaluated the cholesterol biosensor applications of electrodes containing ZnO nanostructures (nanoparticles, thin films, nanowires, nanobelts, nanotubes and nanorods) in terms of electrode architecture and performance [18]. Considering the studies in which cholesterol oxidase (ChOx) immobilization of ZnO based electrodes were carried out, ITO layers have frequently been used in electrode design as well [3, 19–21]. ITO is known as an excellent material widely utilized in biosensor research due to its distinct features, such as high electrical conductivity, robust substrate adhesion, low capacitive current, and consistent electrochemical and physical properties. Furthermore, ITO electrodes are more cost-effective compared to traditional electrodes like gold, silver, and platinum, making them the preferred option [22].

In the study of Umar et al, ChOx was immobilized onto sol-gel derived ZnO nanoparticles. Subsequently, the immobilized nanoparticles were coated on a gold (Au) electrode to create ChOx/ZnO/Au electrode architecture which exhibits a very high and repeatable sensitivity of 23.7 μA $mM^{-1}$ $cm^{-2}$, detection limit (based on S/N ratio) 0.37 ± 0.02 nM, response time less than 5 s, linear range from 1.0 to 500.0 nM and correlation coefficient of $R^2$ = 0.9975 [23]. In another study, Wu et al developed ChOx/ZnO/Ag/GO–CS/ITO electrode in which hydrothermally grown ZnO nanostructures, ChOx, silver (Ag) nanowires, and an indium tin oxide (ITO) electrode with graphene oxide (GO) and chitosan (CS) modification are used. The linear response to cholesterol in the range of the fabricated biosensor was reported as 0.25–400 mg $dL^{-1}$ (6.5 μM to 10 mM) with a sensitivity of 9.2 μA $\mu M^{-1}$ $cm^{-2}$ [19].

In the light of the summarized information, there are many studies on electrochemical cholesterol biosensors with different electrode configurations. In this study, two different electrodes, ZnO-coated ITO/glass electrode (ZnO/Zn/ITO/glass) and platinum (Pt) deposited on ZnO-coated ITO/glass electrode (Pt/ZnO/Zn/ITO/glass), were used for cholesterol detection at different concentrations. ZnO, the most important layer in the biosensor structure, was grown by the electrochemical method, which is a simple method that does not require expensive setup and infrastructure. For the first time in the literature, to the best of our knowledge, the usability of the ZnO structures converted by anodic oxidation of electrodeposited Zn thin films grown on ITO substrates to cholesterol detection was investigated by structural, morphological, and amperometric measurements.

## 2. Materials and Methods

ITO coated glass (ITO/glass) substrates (R<10 Ω/sq) were used as an electrode (Teknoma Inc., Türkiye). In the electrodeposition step, a commercial zinc chloride ($ZnCl_2$) aqueous solution (0.3 M) was used as an electrolyte. The bulk Zn (99.9%) was used as an anode for metallic Zn deposition. Both the electrodeposition electrolyte and the bulk anode were purchased from the metal deposition company (Temeka Metal Coatings Inc., Türkiye). Potassium bicarbonate ($KHCO_3$, Merck, 99.7%) aqueous solution (5 mM) was used as electrolyte in the electrochemical anodization step. For the cleaning procedure acetone ($(CH_3)_2CO$, ≥99.5%, Isolab), isopropyl alcohol (IPA, $C_2H_8O$, 99.9%, Isolab), and distilled water (DI) were used. Also, a self-design sample holder allowing homogenous current flow to substrates with a circular window diameter of 13 mm was designed for metallic Zn deposition and its anodization to obtain ZnO (see Fig. 1). Cholesterol, and Cholesterol Oxidase, Streptomyces sp. purchased and used in biosensor electrode modification (Sigma Aldrich, USA). All substances were employed as purchased and used without any further purification.

### 2.1. Electrochemical deposition of Zn onto ITO/glass

The ITO/glass plates were cut as square formed substrates with a dimension of 2.5x2.5 $cm^2$. The substrates were cleaned in an ultrasonic bath in acetone, IPA, and DI solvents, respectively, for 10 min to remove the remaining contaminants. Afterward, the deposition of metallic Zn was performed in a 0.3 M commercial $ZnCl_{2(aq)}$ electrolyte under 0.1 V constant voltage for 20, 40, and 60 min at room temperature and under 100 rpm magnetic stirring. The Zn deposited samples were denoted as C20, C40, and C60, respectively where C stands for coating process. Additionally, the distance between the anode and the cathode electrodes was set as 10 cm in a two-electrode configuration setup (Figure 1). The Zn/ITO/glass samples were characterized to determine the optimum parameters of the Zn films to be used in the anodization.

### 2.2. Electrochemical anodization of Zn/ITO/glass

The electrochemical anodization process was carried out under 40 V constant voltage and in 5 mM $KHCO_{3(aq)}$ electrolyte for different times (60, 70, 80, and 90 sec) at room temperature in a two-electrode configuration as represented in Figure 1 with Zn/ITO/glass substrates used as the anode and stainless steel as the cathode [16]. After anodization, a heat treatment procedure was applied to anodized samples at 300 °C for 1 hour in air, resulting in the transformation of oxide structures into crystalline structures. The anodized samples were denoted as A60, A70, A80, and A90 to cite anodization duration. After the completion of production steps, ZnO/Zn/ITO/glass samples were characterized.

### 2.3. Characterization

Structural and morphological properties of the deposited and anodized samples were characterized in detail, respectively. The phase structures of the samples were investigated by an X-ray diffractometer (Thermo Scientific ARL X'TRA, XRD) with a Cu-$K_\alpha$ (1.54185 Å) irradiation. Diffraction patterns were recorded at the scan rate of 2°/min in the 2θ range 20°-70° at room temperature. The surface morphology and thickness of the samples were

examined by scanning electron microscopy (Carl Zeiss 300VP, SEM). The micrographs of the deposited samples were taken at magnifications of 10 kX and 25 kX, while 50 kX was used for the anodized samples with a secondary electron detector.

### 2.4. Formation of Electrodes and Cyclic Voltammetry Measurements

As previously mentioned, the electrode architecture and surface quality are one of the critical issues for the formation of bio sensing platform [18]. Due to the fact that samples having the best quality, in accordance with the structural and morphological characterization results, were determined as electrodes to be used in biosensor tests. In addition to the as determined ZnO/Zn/ITO/glass sample, platinum layers were deposited as well for the biosensing performance comparison using a simple approach [24]. Therefore, two samples, ZnO/Zn/ITO/glass and Pt/ZnO/Zn/ITO/glass were considered as cholesterol biosensor electrodes.

Cholesterol oxidase (ChOx) aqueous solutions (1 $mgdl^{-1}$) were prepared at room temperature under vigorous magnetic stirring and coated on the ZnO/Zn/ITO/glass and Pt/ZnO/Zn/ITO/glass electrodes via drop casting method. Subsequent to the ChOx coating, the electrodes were kept at 4 °C for 12 hours. Afterwards the electrodes were washed with 50 mM phosphate buffer solution (PBS) to remove unbound ChOx enzymes [3]. Biosensor tests were conducted by using a three-electrode configuration in which ChOx modified ZnO/Zn/ITO/glass and Pt/ZnO/Zn/ITO/glass electrodes, platinum and Ag/AgCl were respectively used as working electrode, counter electrode and reference electrode to obtain cyclic voltammograms (CV). The 0, 5- and 10-mM electrolytes were prepared by dissolving cholesterol in ethanol/PBS (4:1) solution. CV were obtained at different concentrations (0, 5 and 10 mM) with a scan rate of 50 mV/s between -0.8 and +0.8 V potentials.

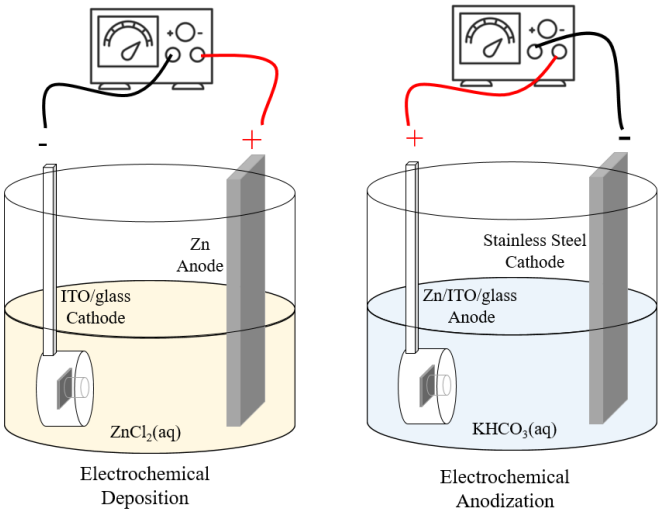


**Figure 1**. Demonstration of electrochemical deposition processes

## 3. Results and Dicussion

The XRD patterns of metallic Zn films obtained by the electrochemical deposition method on ITO coated glasses used as a substrate are given in Figure 2a. When the XRD graphs were examined, characteristic peaks belonging to the cubic ITO phase matching the ICDD 01-089-4598 card number were seen at ≈ 21.8°, 30.7°, 33.3° and 51.2° [25].

In addition, characteristic peaks belonging to the Zn film structure matching the ICDD 00-001-1238 card number were located at ≈ 36.6°, 39.2°, 43.5°, and 54.7° [26]. As expected, it was observed that the peaks of the ITO phase were suppressed by the Zn peaks due to the increasing film thickness as the deposition time increased from 20 to 40 min. Since it was observed that the electrochemical deposition which is applied for more than 40 minutes could not physically adhere to the substrate and peeled off. Therefore, the metallic Zn film process could not be carried out for longer than 40 minutes. As a result, the C40 sample represents the thickest Zn film that can be used for anodizing while maintaining uniformity and good adhesion to the substrate.

Consequence of the structural examination of the samples, the morphology of the C20 and C40 samples were investigated via SEM analysis. Upon examination of the SEM images, it was found that the thickness of the Zn films was approximately 600 nm after 20 minutes of deposition, and approximately 810 nm after 40 minutes of deposition. In addition, it can be seen that the surface is completely covered by the Zn film and the coating is homogeneous according to top-view images (Fig. 2 b-c inset). The examination of the morphology and thickness was hindered due to the peel of the coating observed in the Zn films deposited for more than 40 minutes. Based on the XRD and SEM results, the sample C40 was found to be the most suitable for the electrochemical anodization.

The XRD graphs of the samples subjected to electrochemical anodization for different periods after the electrochemical deposition process were given in Figure 3a. The characteristic peaks belonging to the ZnO structure matching the ICDD 01-079-0207 card number were seen at ≈ 30.7°, and 36.7°. While the Zn characteristic peaks decreased regularly during anodization due to the dissolution of the Zn film and its transformation into ZnO nanostructures, it was observed that the ZnO characteristic peaks continued to form up to 90 sec. After the exceed period from the 90 sec, it was observed that the existing Zn - ZnO structures completely dissolved, leaving only the ITO/glass structure behind.

The SEM images of the anodized samples were shown in Figure 3 b-c. The images demonstrate that dissolution occurs uniformly across the entire film surface of the samples exposed to anodization throughout between 60 and 90 sec. Moreover, it should be noted that the Zn films dissolves and disappears after being exposed to anodization for more than 90 sec. When the anodization time was 180 sec, the Zn/ZnO film structure was destroyed, leaving only the ITO film layer on the substrate. After 300 sec, the ITO film layer also peeled off from the glass substrate due to anodic potential.

As a result of these examinations, it was determined that the most suitable sample to be used in the electrochemical detection of ChOx, was determined as the sample coded A70 owing to obtained structural and morphological properties. Cyclic voltammograms of ZnO/Zn/ITO/glass and Pt/ZnO/Zn/ITO/glass electrodes in electrolytes with various cholesterol concentration were represented in Figure 4a and Figure 4b. When the cyclic voltammograms of ZnO/Zn/ITO/glass were considered the relationship between the current obtained and the cholesterol concentration ranging from 0 to10 mM were found to be linear with an $R^2$ value of 0.9732. The limit of detection the ZnO/Zn/ITO/glass electrode for cholesterol was calculated as 0.98 $x10^{-3}$ M. Similar to Pt free sample a linear relation between cholesterol concentration and the current was observed for Pt/ZnO/Zn/ITO/glass electrode with an $R^2$ value of 0.974 as shown in Figure 4b. Slightly better detection limit was obtained for the Pt/ZnO ITO electrode as 0.965$x10^{-3}$ M.

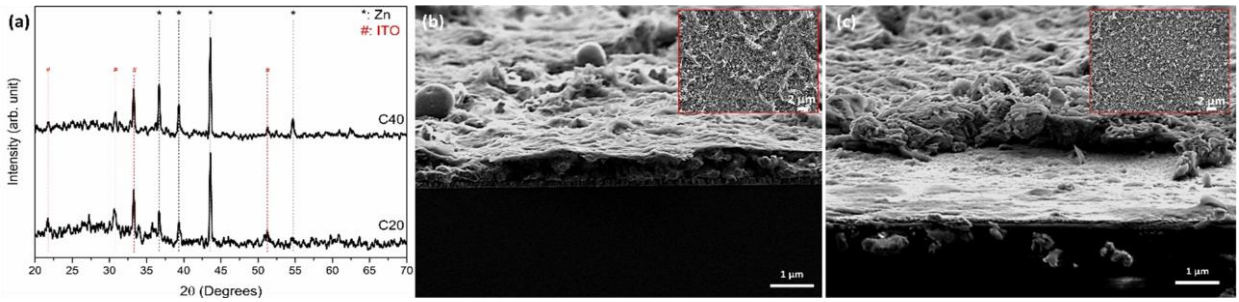


**Figure 2.** (a) XRD pattern of the deposited samples, SEM images of the Zn coated ITO glass for (b) 20 min, (c) 40 min, and the top view images of the samples given in insets.

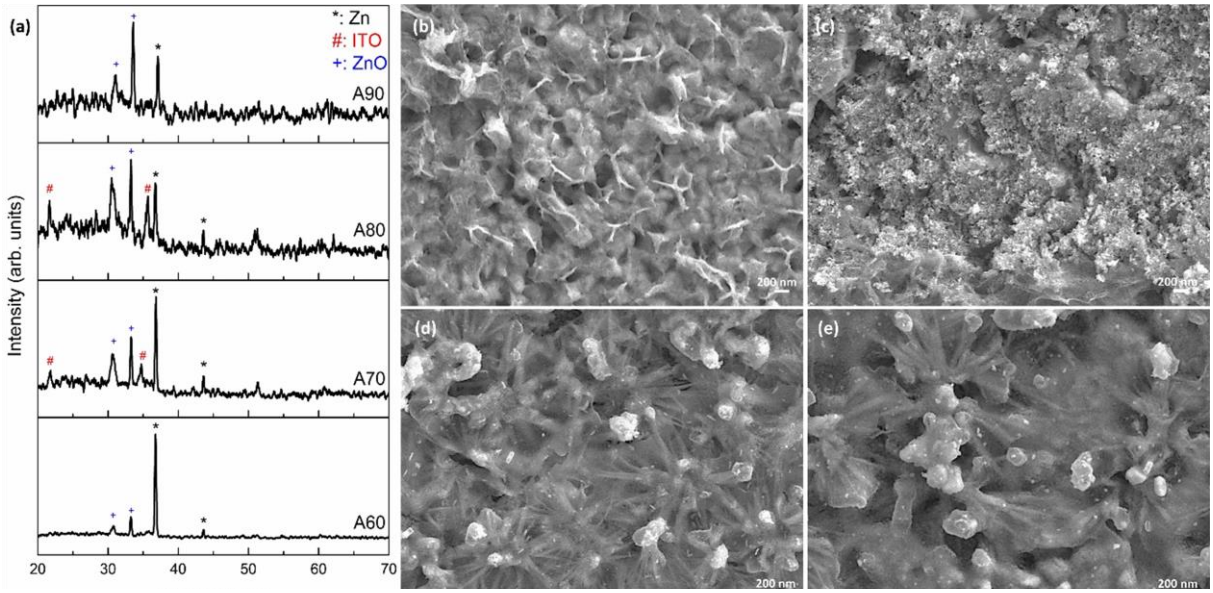


**Figure 3.** (a) XRD pattern of the anodized samples, SEM images of the anodized ZnO nanostructures for (b) 60 min, (c) 70 min, (d) 80 min, and (e) 90 min.

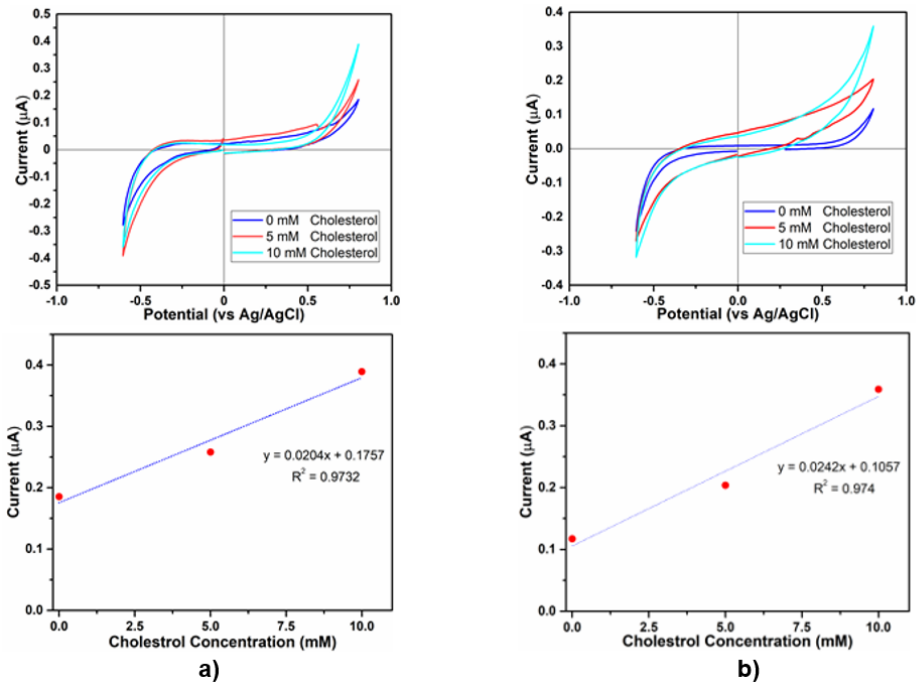


**Figure 4**. Cyclic voltammograms of ZnO/Zn/ITO/glass electrode (a) and Pt/ZnO/Zn/ITO/glass electrode (b).

The cyclic voltammograms results indicates that as developed structures can detect the presence of the cholesterol. Compared to the investigated studies in a recently published review it can be inferred that the obtained results of the present study are promising since the obtained cholesterol detection limits are partly close to the reported studies [18]. Although, in the review of Kumar et al., there are studies denoting the ZnO based biosensors in which the cholesterol detection limits lay between $10^{-3}$ M to $10^{-9}$M levels, the proposed practical way of electrode development process of the study can be considered as a motivation for the development of low-cost biosensor electrodes in which stability and growth of ZnO nanostructure can be elaborately controlled.

## 4. Conclusions

In this study electrochemical deposition and electrochemical anodization processes were successfully performed in order to obtain Zn and ZnO layers respectively on ITO/glass substrates under various processing parameters. As obtained structures were converted in to two different electrodes as ZnO/Zn/ITO/glass and Pt/ZnO/Zn/ITO/glass for the electrochemical detection of cholesterol. The electrodes, prepared for the cholesterol detection, exhibited linear relation between the electrochemical current and cholesterol concentrations of electrolytes. The prepared electrodes had a limit of detection of approximately $10^{-3}$ M, which is relatively high in comparison to the reported results have lower limit of detection in the literature. The results suggest that future studies can develop highly sensitive biosensor electrodes by studying the effect of different types of parameters of low-cost and practical processes on the structural and morphological changes on the surfaces of the electrodes. Apart from the biosensing performance the study proposed a novel approach which does not require expensive infrastructure and raw material cost to develop ZnO based biosensors.

## Ethics committee approval and conflict of interest statement

This article does not require ethics committee approval.

This article has no conflicts of interest with any individual or institution.

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## Author Contribution Statement

M.E. as the project coordinator designed the experiments and required setups together with the assistance of project researchers A.A., M.Y. and T.D.. U.K., B.U. and E.T.Ö. performed the experiments and the characterizations. M.E. took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

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