12-2011

Synthesis of Zinc Oxide Nanorods and use in Biosensor Applications

Anishkumar Manoharan

University of Arkansas

Follow this and additional works at: http://scholarworks.uark.edu/etd

Part of the Biology and Biomimetic Materials Commons, and the Nanotechnology Commons

Recommended Citation

http://scholarworks.uark.edu/etd/232

This Thesis is brought to you for free and open access by ScholarWorks@UARK. It has been accepted for inclusion in Theses and Dissertations by an authorized administrator of ScholarWorks@UARK. For more information, please contact scholar@uark.edu, ccmiddle@uark.edu.
SYNTHESIS OF ZINC OXIDE NANORODS AND USE IN BIOSENSOR APPLICATIONS
SYNTHESIS OF ZINC OXIDE NANORODS AND USE IN BIOSENSOR APPLICATIONS

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Microelectronics-Photonics

By

Anish Kumar Manoharan
Sathyabama University
Bachelor of Science in Electronics and Communication, 2009

December 2011
University of Arkansas
Abstract

The main aim of this research was to develop a nanorod based biosensor for biomedical applications. In this project I use zinc oxide nanorods as the bio-material for biosensor. I fabricated these nanorods using a solution-based technique. Initially I coated a zinc oxide seed layer as the base. This seed layer was then annealed at 350 degrees for almost 1 hour. As a next step, interdigitated electrodes were fabricated on the top of the seed layer using a lift off process. The zinc oxide nanorods were then grown at 90 degrees for almost 4 hours along the electrodes. In this project I have concentrated on making a biosensor for cancer applications, specifically for colon and lung cancer. Cancer specific antibodies, namely Carcinoembryonic antigen (CEA) were immobilized on the top of the nanorods whose effect was identified by measuring the IV characteristics across the electrodes. In this project, I made use the concept of covalent bonding, for which I had a cross linking layer before the immobilization of the antibodies. Nanorods, along with the antibodies, completed the fabrication of the whole sensor. In order to test the sensor, CEA antigens containing the target cells were passed over the device containing the nanorods and antibodies. The capturing of the target cells by the antibodies was confirmed by measuring the IV characteristics across the electrodes.
This thesis is approved for recommendation to the Graduate Council.

Thesis Director:

___________________________________
Dr. Taeksoo Ji

Thesis Committee:

___________________________________
Dr. Sun-Ok Lee

___________________________________
Prof. Ken Vickers

The following signatories attest that all software used in this thesis was legally licensed for use by Mr. Anish Kumar, M. for research purposes and publication.

___________________________________
Mr. Anish Kumar, M. Student

___________________________________
Dr. Taeksoo Ji, Thesis Director

This thesis was submitted to http://www.turnitin.com for plagiarism review by the TurnItIn company’s software. The signatories have examined the report on this thesis that was returned by TurnItIn and attest that, in their opinion, the items highlighted by the software are incidental to common usage and are not plagiarized material.

___________________________________
Prof. Ken Vickers, Program Director

___________________________________
Dr. Taeksoo Ji, Thesis Director
Thesis Duplication Release

I hereby authorize the University of Arkansas Libraries to duplicate this thesis when needed for research and/or scholarship.

Agreed________________________________________

Anish Kumar Manoharan

Refused________________________________________

Anish Kumar Manoharan
Acknowledgements

I would like to thank my professor Dr. Taeksoo Ji for granting me the opportunity to work on this project. I am very grateful for the support and guidance provided by him throughout the course of my Master thesis project.

I sincerely thank Dr. Sun-Ok Lee for her invaluable advice and guidance for successful completion of this project. I am also grateful for her help in providing lab facility during my project period.

I also extend my gratitude to Dr. Simon Ang for his great help throughout my project and also to be kind enough to review my research as part of my graduate thesis committee.

Prof. Ken Vickers has played an immense role in the completion of this thesis and during the course of my master’s degree program. I would like to take this opportunity to express my grateful thanks for all his sincere efforts.

My sincere thanks to my colleague Arun Vasudevan. I have had a number of immensely helpful conversations about various problems during the course of this project and he has patiently guided me through these problems without hesitation.
I would like to thank my family and friends for their support and encouragement in all situations. I would specifically like to thank my father who had motivated to come here and pursue my masters and also has been a great support throughout in achieving my goals.

Research was possible through the use of the High Density Electronics Center at the University of Arkansas, Fayetteville campus. I would like to thank them for their help allowing me to use the equipments inside the clean room in order to complete my project.

My hearty thanks to the Arkansas space grant consortium (ASGC) for funding me and for my project during my Masters project period which was of great support to conduct my project successfully and acquire a graduate degree.
# Table of contents

Chapter 1: Introduction ................................................................................................................... 1  
   1.1 Overview ................................................................................................................................... 1  
   1.2 Significance of the work done ............................................................................................... 5  
   1.3 Overview of the document .................................................................................................... 5  

Chapter 2: Background ................................................................................................................... 7  
   2.1 Literature Review .................................................................................................................. 7  
      2.1.1 Polymer nanowires ......................................................................................................... 7  
      2.1.2 Zinc oxide nanorods ..................................................................................................... 10  
      2.1.3 Aligning of Nanowires ................................................................................................. 12  
      2.1.4 Interdigitated electrodes and immobilization of antibodies ......................................... 13  
   2.2 Need for modification ......................................................................................................... 15  
   2.3 Measurements done while building the system .................................................................. 16  
      2.3.1 Real Time measurement ............................................................................................... 16  
      2.3.2 Cyclic voltammetry ...................................................................................................... 17  

Chapter 3: Synthesis of polymer nanowires and development of gold electrodes for the  
   distribution of nanowires .............................................................................................................. 18  
   3.1 Overview ............................................................................................................................. 18  
   3.2 Synthesis of polymer nanowires ......................................................................................... 18  
   3.3 Fabrication of electrodes and collection of nanowires ........................................................ 21  

Chapter 4: Fabrication of ZnO based biosensor for biomedical applications ............................... 25  
   4.1 Overview ............................................................................................................................. 25  
   4.2 Preparation of zinc oxide seed layer ................................................................................... 25  
   4.3 Fabrication of interdigitated electrodes ............................................................................... 26  
   4.4 Zinc oxide nanowire growth ................................................................................................ 28
4.5 Preparation of sensing part of the biosensor .............................................................. 31
  4.5.1 Surface functionalization of zinc oxide nanorods ................................................. 31
  4.5.2 Antibody ............................................................................................................... 32
  4.5.3 Heavy Chain ....................................................................................................... 34
  4.5.4 Light Chain ......................................................................................................... 35
  4.5.5 CDRs, Fv, Fab and Fc Regions ........................................................................... 35
  4.5.6 Immobilization of biomolecules and testing of the Biosensor ....................... 36
Chapter 5: Result and Discussion ..................................................................................... 39
  5.1 Growth of Zinc oxide nanorods ............................................................................ 39
  5.2 Immobilization of antibody and antigen ......................................................... 41
  5.3 Cyclic voltammetry for binding of antibody and antigen ................................... 44
  5.4 Immobilization of antibody with and without EDC ........................................... 46
  5.5 Sensitivity as a function to with and without antibody ...................................... 47
  5.6 Sensitivity ............................................................................................................... 49
Chapter 6: Conclusion and Future work .......................................................................... 51
References ...................................................................................................................... 53
Appendix A: Description of Research for Popular Publication .................................... 58
Appendix B: Executive Summary of Newly Created Intellectual Property ............... 61
Appendix C: Potential Patent and Commercialization Aspects of listed Intellectual Property Items ...................................................................................................................... 62
  C.1 Patentability of Intellectual Property ................................................................. 62
  C.2 Commercialization Prospects ............................................................................ 62
Appendix D: Broader Impact of Research .................................................................. 63
Appendix E: Microsoft Project for MS MicroEP Degree Plan .................................. 64
Appendix F: Identification of All Software Used in Research and Thesis Generation .... 69
List of Figures

1. Schematic representation of a Biosensor..............................................................3
2. Micromolding process for fabricating polymer nanowires with high aspect ratio........9
3. SEM image of (a) ZnO nanorod grown directly on silicon wafer and (b) ZnO nanorods grown on the top of ZnO seed layer.................................................................10
4. ZnO nanorods grown using ALD process..........................................................11
5. I-V Chr of an aligned single polymer nanowire................................................13
6. SEM image of Wheatstone bridge electrodes fabricated using Lift-off process........14
7. Schematic representation of biomolecules binding using entrapment method.......15
8. Cyclic voltammetry curve....................................................................................17
9A. Top down approach of fabrication of polypyrrole nanowires..........................19
9B. Three electrode cell arrangement.................................................................19
10. SEM image of polypyrrole nanowires resting on the seed layer obtained by just dissolving the alumina template.........................................................20
11. SEM image showing the over etching of the gold electrode..............................22
12. SEM images showing the mixture of un-dissolved alumina templates along with the polypyrrole nanowires...............................................................23
13. SEM images showing Polypyrrole nanowires and little of alumina remains........24
14. SEM image of Zinc oxide seed layer.................................................................26
15. Step by step procedure for fabrication of transducer.........................................29
16. SEM images showing (A, B) well grown zinc oxide nanowires and (C) Zinc oxide nanowires grown along the interdigitated electrodes...............................30
17. EDC chemistry.................................................................................................32
18. Structure of an antibody. ................................................................. 34
19. Schematic representation of sensing mechanism. ................................. 38
20. Cyclic voltammetry curve showing the comparison between the ZnO seed layer and growth of ZnO nanorods. ................................................................. 39
21. XRD pattern for zinc oxide nanorods. ................................................. 40
22. Cyclic voltammetry curve showing the presence of EDC layer. .................. 42
23. Real time measurement showing the addition of BSA after stabilizing the device with buffer followed by the reaction of antigens when captured by the antibodies. .............. 43
24. Cyclic voltammetry curve response for the binding of antibody. ............... 45
25. Cyclic voltammetry curve for measurement of reaction of antigens. ............. 45
26. Cyclic voltammetry response for immobilization of antibody with and without EDC. . 46
27. Real time measurement taken by passing over antigens directly on the ZnO nanowires without immobilizing antibodies. .................................................. 47
28. Percentage ratio for the attachment of antigen with and without antibody. ........ 48
29. Testing of biosensor at two different concentrations. .............................. 50
Chapter 1: Introduction

1.1 Overview

Cancer is one of the world’s largest reasons for human death. We all know that there are hundreds of millions of cells present in a human body. These cells get initiated, multiply and finally die in an orderly manner. In a normal human, as these cells multiply and grow faster the injured cells or dead cells are usually replaced by the dividing of the cells. Cancer cells have a different way of growth when compared to the normal cell growth. These cancer cells grow continuously, spreading the abnormal cells in spite of getting cured. They also attack the other tissues, which is not possible by the normal cells. This kind of act, where cells attack other tissues and have an abnormal growth, is said to be a cancer cell. [1]. There are many kinds of cancer, like lung, breast, prostrate, colon and pancreas cancer, etc. Cancers can cause serious illness and even death if left untreated. As of now there is no cure for this particular disease, but early detection of cancer could be useful in improving the patient’s condition. This can be done with the help of a device such as a sensor that can detect the cancer cells, even at a very minimal level.

A device that has the ability to measure physical quantity of a system and display it as a readable output signal is defined as a sensor. A sensor can also be defined as a device which can receive a signal and respond accordingly. There are three types of sensors namely (a) Physical sensors which can be used to measure temperature pressure, etc, (b) Chemical sensors that can measure the physical or chemical responses according to the chemical substances used, (c) Biosensors which can measure these chemical responses using biological elements. A biosensor
is also a kind of chemical sensor except for the sensing of the biological components like DNA, biomolecules etc.

A biosensor as defined in the Oxford Dictionary of biochemistry is “a device that uses specific biochemical reactions mediated by isolated enzymes, immunosystems, tissues, organelles, or whole cells to detect chemical compound, usually by electrical, thermal or optical signals” [2]. It has two main parts, namely the transducer part and the sensing part. Fig.1 shows the schematic representation of the biosensor that was developed in Dr. Taeksoo Ji’s lab prior to this thesis project, which shows both the transducer and the sensing part of the device. The sensing part of the device is usually exposed to the environment, like the biomolecules in the biosensor proposed in this work. These biomolecules of the biosensor are the ones responsible for the capturing the target cells. A transducer is the heart of the device, which is the base of the system where the sensing biomolecules are attached. This transducer part of the device is the one that transfers the electrons from the biomolecules to the seed, producing a current change which is the indication of its attachment to the transducer. In addition, there is an EDC (1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide) layer that acts as an intermediate layer between the transducer and the sensing part and helps in covalently bonding the biomolecules onto the nanorods. Before the attachment of biomolecules there is a blocking agent, namely BSA (Bovine serum albumin) dropped that helps in grouping the nanorods into groups.

Recently, one dimensional (1-D) nanostructures such as nanowires, nanotubes and nanorods have attracted a great attention among the researchers due to its unique properties and wide range of applications. Due to its hexagonal definition these kinds of nanostructures can be fabricated into a device that possesses high density [3]. These types of structures are the smallest dimension structure, which is an advantage that helps in faster and efficient transport of the
electrons. They are also believed to provide a high performance in terms of sensitivity due to large surface area per unit volume. It is said that in these kinds of 1-D nanostructures electrical properties such as charge accumulation and depletion are seen at a larger proportion due to the reaction of a bulk of structures taking place in a single device. In comparison, in 2-D films, these charge accumulation and depletion takes place only on the surface of the device.

![Fig.1 Schematic representation of a Biosensor](image)

The biomaterials for this work were selected depending on certain criteria like portability, sensitivity and overall performance of the device. In this paper I used conducting polymers and zinc oxide biomaterials, which had many advantages over other biomaterials. They have excellent tunable electrical, magnetic and optical properties. Conducting polymer, especially polypyrrole, has promising applications in the biosensor field due to its biocompatible property and its electrical conductivity is relatively high due to its \( \pi \) conjugated electrons [4-7]. It also has other attractive properties such as the high stability at room temperature and facile polymerization conditions [8]. Another advantage of choosing polypyrrole over other
biomaterials is that it can be grown even at lower potentials (<1V) without creating any changes in the other properties of the material [9-12]. The added advantage of this biomaterial is its flexibility, so that it can be easily implemented into the human body. But I faced a few problems while collecting the nanowires fabricated using polypyrrole, so I switched on to the fabrication of zinc oxide nanowires, which also have most of the advantages as the polypyrrole.

Zinc oxide is a 1-D semiconducting material that has been previously used for several applications such as chemical sensors, photo detectors and FETs (Field effect transistors). It is a II-IV type of semiconducting material which has been most commonly used in electrical and optical applications due to its wide band gap of 3.4eV, allowing easy electron transfer [13] with exciton binding energy at the level of 60meV [14]. Zinc oxide has more advantages when fabricated into specific dimensions than what it possesses as a surface layer. This material has a promising application in the field of biosensors due to its biocompatible property with a high iso-electric point of 9.5. This helps in the immobilization of the biomolecules driven by electrostatic interactions [15, 16]. High electron interactions, large surface area, low toxicity, electrochemically compatible and chemical stability are some of the unique features possessed by the material to be used in the biosensor applications [17].

In this thesis paper I report the fabrication of a sensitive biosensor for the detection of cancer using zinc oxide nanowires that can detect the target cells even at a earlier stage (10ng/ml). Fig.1 shows the sketch of the whole bio-device that was built during the course of the project. I grew zinc oxide nanowires using a solution growth method. Initially I prepared the zinc oxide seed layer using zinc acetate, ethanolamine and ethanol followed by the growth of zinc oxide nanowires on the annealed seed layer using zinc nitrate diluted along with zinc nitrate upto a desired length. I then immobilized the cancer specific antibody on the nanowires using an EDC
cross linker. I later tested the fully built device by passing infected antigens over the sensor, and with the capturing of the cells by the antibody measured by a change in resistance.

1.2 Significance of the work done

Cancer, a contagious disease which can even cause death has no treatment or surgery as of now. But the patient can be medicated and base level treatments can be provided when detected at an early stage. So a sensor which can detect the presence of these cancer cells during a clinical test will be very useful. In this research I built a biosensor that can detect the infected cells even at a very minimal level of 10ng/ml. In this paper I report a biosensor that did detect lung cancer. CEA (Carcinoembryonic antigen) is a kind of biomolecule that is responsible for lung and breast cancer. The level of CEA in a human body can be used for determining the presence of the cancer cells. 0 to 2.5ng/ml of serum in blood is considered to be normal in human who are non smokers. A metastate is said to be reached when the CEA level exceeds 20ng/ml of blood serum. If the level of CEA increases over 10ng/ml indicates the starting stage of cancer. A biosensor that can detect these infected cells below 10ng/ml is considered to be effective for early detection.

1.3 Overview of the document

Chapter 2: In this chapter, I provide the literature survey done during the course of the research. The documents mentioned in this chapter are the ones from which I drew the main idea of the research project.

Chapter 3: In this chapter, I explain the previously done work of synthesis of polypyrrole nanowires and the fabrication of the Wheatstone bridge electrodes using an etching method. At
the end I briefly explain the problems I faced during the project and the need to switch on to a new work.

Chapter 4: In this chapter, I explain methods and the procedures used in fabricating a biosensor step by step. The chapter starts with the synthesis of zinc oxide nanowires followed by the fabrication of the electrodes using lift off process. My brief explanation about the biomarker and the carcinoembryonic antigen is followed by the technique used for the immobilization of the biomolecules. Finally I explain the steps used for testing the biosensor.

Chapter 5: In this chapter I focus on providing the results obtained during each process with a brief explanation.

Chapter 6: In this chapter I describe my conclusions and projected future work.
Chapter 2: Background

2.1 Literature Review

The fabrication of biosensors using polymer or zinc oxide nanowires has been done from late 80’s time. But the method of fabrication keeps changing from time to time to impact considering certain main parameters such as cost, time taken to fabricate, improvement of sensitivity and many others. In this chapter I will be discussing about some of the previous methods that were used to fabricate polymer nanowires, zinc oxide nanowires and the method that was used to immobilize the biomolecules onto the nanowires. Finally I will briefly describe the need for an improved design and the parameters measured at each stage of fabrication of my device.

2.1.1 Polymer nanowires

Yen Wei. et. al., and his group, in 1991 were the first people to produce polypyrrole powder and coating the polymer as a thin film layer [18]. This caught the interest of many scientists who tried to do research using polymers because of wide availability of the material and the ease of use of the material. Later this material was used widely for various applications. This material as mentioned earlier was coated as an initial layer. As the technology developed it was fabricated in the form of 1-D nanostructures, mainly as nanowires. They were fabricated using slip cast and sol gel method, but recently fabrication of this material is done using alumina templates that are commercially available. Previously these alumina templates were prepared using photolithography process, but template holes at nanowire sizes cannot be prepared using a photolithography process.
The polymer nanowires were fabricated using various methods like the track etch method, printing techniques, photochemical lithography, etch lithography and micro-molding technique. In the micro-molding technique, first a mold made of silicon nanoposts was prepared using photolithography process in a bottom-up approach [19]. Then an anti-sticking PDMS layer was coated on the top of the mold for hours and then peeled off. The peeled off PDMS anti-sticking layer can be seen in the Fig.2E. This peeled off layer containing the nanowire sized holes was then filled with the polymer liquid along with a liquid metal and cured. The strengthened nanowire layer was then removed which could be used for further process. This procedure was a very time consuming process, and various other problems such as adhesion forces between adjacent nanoposts and surface morphology to bear the posts were a big problem when the nanowires were fabricated so they started fabricating the nanowires using a three electrode cell method.

To use this electrochemical process they had to prepare alumina templates having hollows of a certain diameter and length so the process of anodic oxidation was followed by making templates of high electrical and thermal stability [20]. An alumina template with a purity of 99.8% was prepared. These had a high pore density and uniform pore diameter when compared to the ones prepared before them, but these alumina templates faced the issue of high temperature. It is said that the templates did not react with the liquid at high temperature of about 80°C and due to this the electrical resistance of the device was in an uncontrolled state. Adding to this problem, the process of preparing the alumina template was very long and time consuming and the pore size of the templates could be fabricated only at micro level and cannot be fabricated at a nano range level. Recently alumina templates that are commercially available are used, which are available at nano size pore levels.
Fig. 2 Micromolding process for fabricating polymer nanowires with high aspect ratio, (A) SEM image of Silicon master bearing square array posts grown using photolithography process, (B) Silicon master with liquid PDMS, treated with antisticking agent, (C) Cured PDMS peeling off from master, (D) Negative PDMS containing high aspect ratio hollows, (E) SEM image of hollow negative PDMS mould, (F) Polymer and liquid metal being cured in the mould, (G) Negative PDMS peeled off from actual polymer nanowire, (H) SEM image of nanostructured replica fabricated from epoxy resin. [19]
2.1.2 Zinc oxide nanorods

In 1996, Peulon. et.al., was the first to develop zinc oxide nanorods using an electrodeposition method [21]. They proposed that the zinc metal could not be deposited directly onto the substrate and that it had to be oxidized. Before growing the nanorods, a seed layer was formed on the substrate. This seed layer acted as a base for the nanorods to grow. There are various methods by which this seed layer could be coated, and one of the method followed is the atomic layer deposition (ALD).

Fig.3 SEM image of (a) ZnO nanorod grown directly on silicon wafer and (b) ZnO nanrods grown on the top of ZnO seed layer [22]

The purpose and the effect of growing the nanorods on the seed layer was proved by testing the growth of zinc oxide nanorods on a silicon wafer and on the top of the zinc oxide seed layer. The SEM image in the Fig.3 shows the comparison that was tested. It can be seen that the nanorods grown on the bare silicon wafer have not been distributed well and there are gaps present between groups of nanorods [22]. But the nanorods grown on the zinc oxide seed seemed
to be well organized and have organized distribution of nanowires. The ALD process used for
the deposition of this seed layer seemed to be a bit complexed and a more time consuming
process than the one reported in this paper.

In one of the recent papers, it was reported that the zinc oxide nanorods were grown
using vapour solid process. In this method, a certain amount of zinc oxide powder in mixture
with the carbon powder was placed on an Al$_2$O$_3$ boat and loaded into a horizontal high
temperature furnace tube along with the substrate on which the nanorods were to be grown [23].
The furnace was equipped with a gas controlling system and a rotary pump. The reaction was
allowed to take place at a high temperature of 1400$^\circ$C for two hours. This method was not a
stable one and the nanorods that were formed using this method were not well aligned as seen in
the SEM image in Fig.4.

![Fig.4 ZnO nanorods grown using ALD process [23]](image_url)
2.1.3 Aligning of Nanowires

The nanowires that are synthesized electrochemically will be distributed randomly over the surface of the electrodes that are fabricated on the top of the wafer. The dispersed nanorods will be present at any angle, which then have to be aligned across the electrodes in order to immobilize the proteins on them. The advantage and need for aligning the nanowires is to make way for attachment of proteins on the maximum number of sites on the nanowires in a way to improve the sensitivity of the device. If the nanowires are not aligned, there is a chance of overlapping of the nanowires which would affect the possibility of attachment of proteins onto that site. For this aligning of nanowires, dielectrophoresis method has been widely used by many researchers since the nanowires get aligned very effectively along the electrodes.

The other main reason for using dielectrophoresis for aligning the nanowires is that large amounts of nanowires can be aligned at the same time and also can be tested very easily. Before proceeding on to the aligning of nanowires, the wafer is patterned with the electrodes design using chromium and gold. Chromium is used just for the attachment of the gold metal onto the silicon surface. This patterning of electrodes can be done either by a wet etch method or by using a lift off process. The lift off process is more effective to obtain electrodes with sharp edges when compared to that of the wet etching process.

According to [24], the polymer nanowires they created were removed from the substrate using sonication and were suspended in the ethanol solution. The polymer nanowires presence was realized by applying a potential along the electrodes where the current along the electrodes were also measured. Fig.5 shows I-V curve that was measured during the dielectrophoresis method. According to [25], 5Mhz frequency at a constant potential of 1V was applied along the electrodes where the nanowires were believed to be aligned due to applied electric charges. But
the dielectrophoresis method can be done only when the nanowires are in contact with the ends of the electrodes.

![I-V characteristics of an aligned single polymer nanowire](image)

Fig.5 I-V characteristics of an aligned single polymer nanowire [25]

### 2.1.4 Interdigitated electrodes and immobilization of antibodies

After the deposition of the zinc oxide seed layer, the most important and necessary part that has to be done is the fabrication of the electrodes before the growth of the nanowires. These electrodes are fabricated in the form of fingers of two hands crossed each other (Fig.14) instead of just having parallel electrodes. The sensitivity of a sensor also depends in the way these electrodes are designed. Usually these electrodes are fabricated using etching process where the photolithography process is done after the deposition of the metal. This metal is then etched using a corresponding etchant. But the main disadvantages of using an etching process for the fabrication of the electrodes is that the etch rate has to be controlled for the electrodes to have
uniform pattern that does not expose underlying chrome. Over etching of the material may also result in non-uniform pattern of the electrodes.

![Fig.6 SEM image of Wheatstone bridge electrodes fabricated using lift-off process](image)

After the fabrication of the electrodes and the growth of the nanorods, the next and the final part of my fabrication of the sensor was the immobilization of the antibodies onto the nanorods. In this part I concentrated on how effectively the antibodies could be attached to the nanorods. The method I proposed resulted in the antibodies being firmly inserted into the nanowires irrespective of whether the nanowires were standing up vertically or lying down horizontally on the base. Earlier, entrapment method was used for immobilization of the antibodies. Fluorescence material, FITC was attached onto the antibody, biotin, and then was immobilized on the nanowires by just dropping the biotins over the nanowires [26]. A change in signal was measured externally during the attachment of the biotins onto the nanowires. The
main disadvantage of this method was that the antibodies just got attached with the nanowires and not strongly imbibed into it. It was also reported in the same paper that the due to this only less number of biomolecules got attached to the nanowires. The schematic representation comparison of attachment of the biotin to the nanowires and attachment without nanowires can be seen in the Fig.7.

![Fig.7 Schematic representation of biomolecules binding using entrapment method][24]

### 2.2 Need for modification

The main modifications I studied in this work are the method in which the nanowires were grown and the method by which the antibodies were immobilized. I did these in order to improve the sensitivity of the sensor with a reduced amount of fabrication time. Although having
less time consumption for the fabrication of the device was a secondary challenge, the main challenge was to improve the sensitivity of the device so that the target cells could be easily captured, even at a very minimum level of 10ng/ml. I propose in this work that the growth of zinc oxide nanowires using solution based technique is an easier and time consuming method than the one mentioned in the review. I used the method of cross linking for the firm attachment of the antibodies onto the nanowires which led to attachment of antibodies upto 20ng.ml concentration onto the surface, improving the sensitivity of the device. The sensitivity of the device was also measured by the response time taken by the antibodies to capture the target cells.

2.3 Measurements done while building the system

2.3.1 Real Time measurement

I did real time measurement so that there is less noise interference and also so that the immobilization of proteins took place in a proper manner [27]. I did the real time measurements at a fixed voltage in order to measure the response time and the current passing through the device while the binding of any material took place. I used a Keithley instrument for my real time measurements at a constant voltage of 2V. I did the measurement for about half hour continuously for the process of immobilization of antibody, addition of buffer, addition of BSA (bovine serum albumin) and the immobilization of antigen on to the antibodies. This real time measurement was also very useful in measuring the specificity of the sensor which was tested by immobilizing the antigens directly on the nanowires.
2.3.2 Cyclic voltammetry

In general, the electrochemical analysis of an analyte is done using cyclic voltammetry curve. As the name sounds the reaction occurs in a cycle manner where the potential increases in a linear fashion from the initial voltage to the applied maximum voltage along the electrodes at a fixed rate. This fixed rate is called the scan rate where the voltage is swept step wise at a constant range. Current passing through the electrodes is measured along the working and the counter electrode by applying a potential across the working and reference electrode. This behavior of applying voltage from the minimum voltage to the maximum voltage is similar to the linear sweep voltammetry [28]. The voltage then sweeps back from the maximum voltage to the initial voltage. The analyte is said to be reduced or oxidized during the forward scan and re-oxidized or re-reduced during the reverse scan [29]. In this paper I used cyclic voltammetry curves for oxidation or reduction that occurred during the binding of the antibody and antigen.

Fig.8 Cyclic voltammetry curve [29]
Chapter 3: Synthesis of polymer nanowires and development of gold electrodes for the distribution of nanowires

3.1 Overview

Polypyrrole, a conducting polymer, is preferred over other polymers for fabrication as a biosensor due to its high material stability and low electrical conduction. The material stability property of the polypyrrole helps in fabrication a biosensor with high sensitivity in comparison with biosensors fabricated by other materials. Earlier the monomer pyrrole was prepared using oxidation process and was a black colored powder, leading to it being named as “pyrrole black” [30]. This pyrrole was later synthesized as a continuous thin layer, which led to the material being adopted by researchers for various applications. Polypyrrole has become a very promising material in the field of biosensors at recent times [31].

3.2 Synthesis of polymer nanowires

The fabrication of nanowires with a particular aspect ratio was one of the major challenges. My fabrication of the polypyrrole nanowires was done using a top down approach as shown in the Fig.8. I made use of an alumina template which had pores in the form of a cylinder that were commercially available. Initially I coated the backside of the alumina template with approximately 100nm of silver metal to cover the pores. This silver metal acted as a seed layer on which the polypyrrole nanowires were grown using a three electrode method as shown in Fig.9 [32].
Fig. 9A Top down approach of fabrication of polypyrrole nanowires

Fig. 9B Three electrode cell arrangement
The three electrode setup has three electrodes, the working electrode, counter electrode and reference electrode. The working electrode is the sample in which the nanowires are to be fabricated, and in my project it was the alumina template with a seed layer at the backside. The counter electrode was a platinum mesh and the reference electrode was an Ag/AgCl rod. The electrodeposition of nanowires was done using a potentiostat purchased from Gamry Instruments REF 3000. The deposition of the nanowires was done using various conditions and I found that the nanowires were obtained at a potential of 0.065V with a sweep rate of 0.075mV/sec for 1 hour. Fig. 10 shows the SEM images of the polypyrrole nanowires grown up to 5µm in length which were obtained by dissolving the alumina template using 5M NAOH solution with the seed layer still present at the bottom. I dissolved the only the alumina templates without etching the seed layer in order to see whether the nanorods actually get formed or not.

Fig.10 SEM image of polypyrrole nanowires resting on the seed layer obtained by just dissolving the alumina template
3.3 Fabrication of electrodes and collection of nanowires

I prepared interdigitated electrode in the form of Wheatstone bridge (Fig.14) using evaporation on a SiO2 wafer. Initially, I cleaned the SiO2 wafer with acetone, IPA and distilled water and mounted the wafer inside Edwards Evaporator to coat the gold metal. An adhesion layer of chromium was coated up to 10nm and the gold metal was coated approximately 100nm. The metal deposition is followed by photolithography process to pattern the Wheatstone bridge electrodes using photo-resist. I did negative photolithography process using a bright field mask. The metal on the portion where the photoresist were absent was etched out leaving behind the electrodes design. I etched the chromium and the gold using CEP 200 chromium etchant with an etch rate of 4nm/sec and GE8148 gold etchant with an etch rate of 20nm/sec.

Fig.11 shows an SEM image of electrodes that I fabricated using an etching process. I observed that the middle part with the nanowires was the wafer part, and the electrodes were present at the sides. It can be observed that I overetched the gold layer resulting in decrease of the gap size between the electrodes. This resulted in contact of the nanowire with the chromium layer, which resulted in electrical property change due to those contacts. The other main factor that had to be corrected was the pattern design, which affected the sensitivity of the sensor. The length of the electrode and the gap between the electrodes played a major role in improving the sensitivity. I later washed the template containing the nanowires with distilled water and then I etched the seed layer at the backside of the template leaving behind only the alumina template and the nanowires.

I then dissolved the template using 5M NaOH solutions leaving behind the nanowires in the solution. I followed two different ways to collect the nanowires. The first way was a filtering method in which the NaOH solution containing the nanowires was filtered using a polycarbonate
filter paper. I found that even after filtering two to three times, large amount of alumina was not dissolved completely. These undissolved thick templates were found to block the view of the nanowires when dropped on the top of the electrodes. The SEM image in Fig.12 shows the partially dissolved alumina templates along with the nanowires.

![SEM image showing the over etching of the gold electrode](image)

**Fig.11** SEM image showing the over etching of the gold electrode
Fig. 12 SEM images showing the mixture of un-dissolved alumina templates along with the polypyrrole nanowires
Since the problem in the first method was the undissolved templates, I switched to an alternative method in which I could separate the alumina particles from the nanowires. So I chose the centrifuging process. This process works using the concept that when a solution containing particles of different sizes is spun at a certain speed at a certain angle, it will make the smaller particles that are light weight float on the top leaving behind larger particles at the bottom. So I spun the NaOH solution containing the un-dissolved alumina templates and the nanowires at 10,000 RPM. Approximately 1ml of the solution from the top was collected separately and the nanowires in the solution were washed by rinsing it with with DI water upto 2 times. Believing that the polypyrrole nanowires that are light weight would be floating on the top, I dispensed the solution on the top on the fabricated gold electrodes. From the SEM image in the Fig.13, it was observed that I was not able to completely eliminate the alumina templates.

![SEM Image](image.png)

Fig.13 SEM images showing Polypyrrole nanowires and little of alumina remains
Chapter 4: Fabrication of ZnO based biosensor for biomedical applications

4.1 Overview

Due to its unique physical and chemical properties, zinc oxide material is believed to create a breakthrough in the field of sensor technology and mainly the biosensors field. The zinc oxide material is used in various applications such as Piezo-electric devices [33-35], photo electrochemical applications [36-39], optoelectronics [40, 41], Solar cells [42-45], LED [46] and gas sensors [47]. Recently, the use of this material in the biosensors field has been widely used due to its promising applications. There were various methods to grow zinc oxide nanorods that were proposed by scientists [48-50], but they required expensive equipment or the process were very time consuming compared to the method we have proposed in this thesis. Here, I have proposed fabrication of zinc oxide based biosensor using a solution process based technique, which could be used for biomedical applications like cancer. In this chapter, I have explained each step of fabrication of the biosensor and the theory behind its operation.

4.2 Preparation of zinc oxide seed layer

The preparation of the zinc oxide seed layer was the first step in fabricating the transducer part of the biosensor. I initially cleaned the silicon wafer for coating the seed layer by sonicating it with acetone, isopropyl alcohol and distilled water. The procedure for forming a zinc oxide seed layer was followed as explained elsewhere [51]. I prepared the seed layer solution containing 0.1M zinc acetate and 0.1M ethanolamine in ethanol by stirring the content at 70°C for 1 hour. Later I spun the solution on the silicon wafer at 1000 RPM for 20 seconds and was heated at 170°C for 5 minutes for the wafer to dry. I repeated the procedure for 5 times to get a uniform seed layer throughout the wafer and annealed the seed layer with the wafer at
350°C for 4 hours. While annealing at this high temperature, the zinc acetate decomposed and zinc oxide formed due to the reaction of oxygen with zinc in presence of air. Fig.14 shows the SEM image of the zinc oxide seed that was obtained after annealing process.

![SEM image showing Zinc oxide seed layer]

**4.3 Fabrication of interdigitated electrodes**

The next step was the fabrication of the interdigitated electrodes on the top of the seed layer. I fabricated the electrodes using a lift off process. This process was followed so that I could get uniform electrodes with sharp edges in comparison to the electrodes that were obtained using etching process. In a lift off process, the two main procedures are photolithography and the evaporation of the metal. The photolithography process involves four main steps, namely coat, bake expose and develop respectively.
I coated a layer of resist upto 1.8µm thickness at 2000 RPM for 60 seconds on the top of the seed layer, followed by heating it at 100°C for 2 minutes, and cooling it for 30 seconds. I then exposed the wafer to UV light with the mask bearing the electrodes design. Since I had to do positive photolithography, I used a dark field mask which there was clear at the place of the electrode design for the UV light to pass through and depolymerize the resist. To remove the photoresist present at the places of the electrodes, I immersed the wafer in a developer solution. The wafer was then thoroughly cleaned and dried to deposit the metal.

I evaporated the gold metal using Edwards evaporator with a thin layer of chromium acting as the adhesion layer. A gold layer of 100nm thickness was evaporated on the chromium surface of thickness 8nm. The wafer was then sonicated for about 2 minutes in an acetone solution which dissolved the photo-resist, removing the chromium and gold material on the places other than the original electrodes where the metals were deposited directly on the seed layer. The metal pattern remaining formed a Wheatstone bridge. The design had four terminal ends, each leading to inter-digitated finger-like electrodes that are interconnected together representing a Wheatstone bridge as seen in Fig.6.

A Wheatstone bridge works in the concept of a voltage divider where the bridge is said to be balanced when the current passing across the resistors are the same. In a basic Wheatstone bridge, a DC voltage is applied to the circuit and current is measured to check the balance of the circuit. Even a small change in resistance can be measured with a voltage excitation source and if a change occurs due to some environmental factors like temperature, pressure etc can also be measured in the Wheatstone bridge. The Wheatstone bridge circuit which is said to be balanced will compensate the offset of all the resistors connected in the circuit. In recent times, the Wheatstone bridge concept has been mainly used for developing sensors for various applications.
4.4 Zinc oxide nanowire growth

The next step was the growth of the zinc oxide nanorods. Hexamethylenetetramine and zinc nitrate were used for zinc oxide nanorod growth as described in [52]. 0.025M of zinc nitrate and 0.025M of hexamethylenetetramine was mixed with 80ml of distilled water for about 2 hours at room temperature. I immersed the wafers containing the zinc oxide seed layer with the electrodes in to this solution mixture and aged it in the oven at 350°C for approximately 4 hours. Fig.15 shows the step by step process by which the biosensor device was fabricated. It can be seen that the seed layer was first spin coated followed by the deposition of metal in the form of Wheatstone bridge. As a final step I grew the zinc oxide nanorods on the top of the seed layer. One gap between the electrode fingers is shown in the schematic in Fig.15 where the growth of the nanorods in between the electrodes is represented diagrammatically. The sample containing the nanorods was then cleaned with the acetone solution and sonicated for about 2 minutes in order to remove the particles that were present due to the solution mixture. Well grown zinc oxide nanorods were seen on the silicon wafer through the SEM with a length of about 500nm and a diameter of 60-100nm. Fig.16 shows the SEM images of the zinc oxide nanorods that were grown in between the electrodes. It can be seen that the nanorods are grown in between the electrodes and also that the growth was absent at the edges of the electrodes. This might be due to the non-exposure of the solution with the seed layer part at those places. The zinc oxide nanorods along with the electrodes make up the transducer part of the biosensor that was ready for immobilization of biomolecules on the nanorods.
Fig. 15 Step by step procedure for fabrication of transducer
Fig. 16 SEM images showing (A, B) well grown zinc oxide nanowires and (C) Zinc oxide nanowires grown along the interdigitated electrodes
4.5 Preparation of sensing part of the biosensor

4.5.1 Surface functionalization of zinc oxide nanorods

Functionalizing of the nanowires means to change its chemical property and prepare it to covalently bond with the antibodies. The functionalization of nanowires can be done in two different ways, namely entrapment method and covalent attachment. But recently covalent attachment of biomolecules over entrapment method [53] has been widely accepted and followed by many researchers. This is because the entrapment method has adverse synthesis conditions during the biological activity of the biomolecules, and also that the biomolecules get buried deep into the nanowires which will lead to a decrease in the sensitivity of the device and to an increased response time. It has also been stated in [53] that the biomolecules get attached onto the surface of the cross linker in such a way that the biomolecules is readily available to capture the infected cells, increasing the sensitivity of the sensor. The cross linkers are the ones which acts as an intermediate bonding layer between nanorods and the biomolecules. In the covalent attachment of the biomolecules there are two types of linkers that can be used, namely the glutaldehyde and EDC (1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide) [54]. In this paper [54], a comparison between using both of these cross linkers was tested. The two cross linkers were in separate samples and immobilized the antibodies of same concentration along with the FITC (Fluorescein isothiocyanate), a fluorescent tracer used for identifying the amount of biomolecules attached on the nanorods. It was found that the fluorescence intensity due to the attachment of antibodies was less in the sample containing glutaldehyde in comparison to the intensity obtained in the sample containing EDC. So I made use of an EDC cross linker for the activation of the nanorods surface to prepare it for the immobilization of the antibodies.
Fig. 17 shows the diagrammatic representation of the EDC chemistry that takes place when the EDC/NHS (1-Ethyl-3-(3-dimethylaminopropyl)carbodimide/N-hydroxysulfosuccinimide) covalently binds with the nanorods. The EDC layer gets attached to the ZnO surface with the help of the hydroxyl groups present on the surface. The reaction of NHS forms an amine bond NHS ester increasing the coupling efficiency even though can be done even without NHS.

![EDC Chemistry Diagram](image)

Fig. 17 EDC Chemistry

4.5.2 Antibody

An antibody, a large Y shaped protein which is also known as an immunoglobin, can be used to detect the foreign cells and neutralize them if found in the immune system. This antibody can only be used to find its own specific antigen to neutralize. The antigen-antibody works with the concept of lock and key. Each antibody that is Y shaped will have an paratope, which is considered to be the lock to their specific epitope, which is the key that allows both of them to be attached or bind together effectively. The humoral immune system in the body is the main factor that is responsible for the production of antibodies.
An antibody has two physical forms namely; soluble form and membrane bound form. The soluble form is the one that is secreted from the cell, whereas the membrane bound form is directly attached to the b cell surface which is named as B cell receptor. Present on the surface of the B cell, these BCRs are facilitated and activated into either antibody factories that form the plasma cell or as a memory B cell that will remain in the body along with its specific antigen so that the B cells can be useful to respond quickly during future exposure. In many cases, the full activation of the B cell occurs due to the interaction between B cell and the helper T cell which in turn helps in the antigen binding to the antibody that is generated.

As seen in Fig.18, antibodies have two large heavy and light chains. This is the basic structure of any antibody, but there are different kinds of heavy chains present for an individual antibody. They are grouped into various isotypes depending on the type of heavy chains that they posses. The base of the heavy chain is switched by re-organizing the antibody genes which helps them in attaching to their specific antigen. This is how difference parts of the immune systems are made to attach with a single kind of antibody.
4.5.3 Heavy Chain

The mammalian immunoglobulin heavy chains are of five types namely α, δ, γ, μ and ε which are found in IgA, IgD, IgG, IgM and IgE respectively. The types of heavy chains mentioned above defines the class of the antibody. The heavy chain’s size and composition depends on the number of aminoacids present in it. For example, α and γ contain approximately 450 amino acids whereas μ and ε contain approximately 550 aminoacids. The two parts of the heavy regions as seen in Fig.18 is named as the constant region and variable region. Heavy chains have a constant region and a hinge region for added flexibility. Heavy chains having a constant region contain four immunoglobulin domains. Variable region has different antibodies that are produced by either a single or a multiple B cells.
4.5.4 Light Chain

In a light chain there are two types of immunoglobin namely lambda (λ) and Kappa (κ). Since the light chain is present at the base of the antibody, they have only two domains namely constant domain and variable domain. The length of the light chain in an antibody is usually 211 to 217 amino acids.

4.5.5 CDRs, Fv, Fab and Fc Regions

An antibody also has some parts which have unique functions. In the Y shaped part of the antibody there are sites which can bind two antigens onto it which is useful in recognizing the foreign specific antigen objects. The fab (fragment, antigen binding) region has a constant and a variable domain. They are present in both the heavy chains and light chains. The most important region for the attachment of the antigens to the antibody is the Fv region which is called as the variable domain. In particular, there are three light and heavy chains on the β strands which is responsible for the binding of the antigen. These chains are present in the form of loops which is named as complementarity determining regions (CDRs). Chothia et. al., [55] and North et. al., [56] have well studied these CDRs and have classified their structures briefly. According to immune network theory, CDRs are named as idiotypes where the immune system is regulated due to contact between these idiotypes. The Fc (Fragment, crystallizable) region, which is the base of the Y shaped antibody, is the main region that modulates the immune cell activity. Depending on the class of the antibody, they have two heavy chains that contain two or three constant domains.
4.5.6 Immobilization of biomolecules and testing of the Biosensor

The aim of this project was to build a biosensor for cancer applications. So I chose the carcinoembryonic antigen (CEA) which is specific to lung cancer. The successful immobilization of this CEA antibody onto the nanowires completed the fabrication of the biosensor. Readily available CEA was purchased from sigma Aldrich. This CEA was mixed with the tris phosphate buffer solution \( (1 \times 10^4 \mu g/ml) \) so that the antibody was in a good condition for a long period of time. The attachment of the biomolecules was realized using cyclic voltammetry method. A brief explanation of this method was described in chapter 2. I used the same concentration of \( 14 \times 10^2 \mu g/ml \) antibodies throughout the project while testing the biosensor each time.

After the addition of antibodies, a 2 micro liters of buffer solution were added to bring the sensor to a stable condition. Then the blocking agent, namely BSA, was added to the device and deposited between groups of nanowires containing the antibodies for attachment of antigens on the un-immobilized antibodies. As a final step, the antigens at a concentration of 10ng/ml containing the target cell were dropped on the sensor for capture. The biosensor was also tested using a higher concentration of 20ng/ml to prove the sensitivity of the device as a comparison. The biosensor was also tested by dropping only the antigens on the nanorods without immobilizing the antibodies.

The mechanism of sensing or the capturing of the antibodies and the antigens was done in the following way. The antibody and the antigens have a specific charge depending on the iso-electric point. In [57], it is said that the pH of a biomolecule carries does not have any net charge, but it will possess a net positive charge below its iso electric point and a negative charge above the iso electric point. The purification of a protein can be determined by its iso electric point in accordance to its pH whose solubility is minimal and mobility in an electrofocusing system is
zero. It is said that proteins have a negative when the pH value is higher than the pI value [57]. It is also said that the CEA posses a negative charge due to its acidic nature at a pH of 7.4, which results in change on the liposome surface depending on the concentration of CEA. For my project, I have used CEA which had a pH of 7.5, which proved that the proteins used had a negative charge.

Under optimized conditions, the electrochemical response of the antibody and the antigen were recorded. The electrochemical response showed an immediate increase in the current and then after some time a stabilized current was able to be observed. A similar kind of response was seen after the addition of the antigen, but at this time there was a sudden decrease in the current followed by a stable current at the end. The stable current indicates that all the biomolecules have got settled. This kind of reaction was also observed when only the antigens were added without the antibodies. The response due to the addition of the antibody will not be the same as the reaction obtained when both the antibody and the antigen are present. Although there is no specific sensing mechanism for the biological detection of biomolecules like antibody and antigen, in [58] the sensing mechanism is explained for DNA detection on the ZnO thin films. The same mechanism can be applied for the antibody-antigen reaction on the ZnO nanorods reported in this paper. It is explained in the paper [58] that when a DNA strand attaches to the ZnO film, the negative charge of the DNA will influence the spatial charge present on the electrodes thereby repelling the electrons in the ZnO which is an n-type semiconductor. Due to this, the conduction band edge curvature moves towards positive energies leading to electron density reduction in the spatial charge zone.

In reference to it, I propose two possible mechanisms for the working of the biosensor during the addition of antibody onto nanowires and also during the addition of antigens onto the
antibodies. Firstly, I propose that the electrons may flow directly through the nanowires due to the interactions between the adjacent nanowires as shown in Fig. 19. So when the negatively charged antibody attaches to the n-type semiconductor ZnO nanorod, the electrons in the nanorod may repel due to the negative charge of the antibody forming a depletion layer on the nanorod. This leads to decrease in conductivity therefore an increase in the resistance. Secondly, the electrons may flow from the nanorod to the seed layer on which the nanorod is resting and then reach the electrode. In this possible chance, the depletion layer gets extended to the seed layer which in turn leads to the decrease in the conductivity and an increase in the resistance on the ZnO nanorod and the seed layer.

Fig. 19 Schematic representation of the sensing mechanism
Chapter 5: Result and Discussion

5.1 Growth of Zinc oxide nanorods

The presence of zinc oxide nanorods was detected by using a current voltage (IV) curve. Fig. 20 shows the comparison between the IV curve for the presence of the zinc oxide seed and the zinc oxide nanorods. The solid line curve indicates the IV curve for seed layer and the dotted line curve indicates the IV curve of the ZnO nanorod. Initially, the current that flows due to the presence of seed layer was obtained followed by the IV measurement after the growth of the nanorods on the seed layer. It can be seen that the current increased due to the growth of the nanorods as the number of electrons has increased but the exact linear response was not obtained for which the reason is unknown. This assured the growth of nanorods on the top of the seed layer.

Fig. 20 Cyclic voltammetry curve showing the comparison between the ZnO seed layer and growth of ZnO nanorods
The characterization of zinc oxide nanorods was done by using XRD spectrum. Through XRD spectrum I analyzed the structure and the orientation of the nanorods. Fig.21 shows the XRD pattern of the zinc oxide nanorods that were grown using the solution process technique which was analyzed using a Rigaku X-ray Diffractometer. The standard data of ZnO (ICDD #00-036-1451) was matched with the nanorods grown which told me that the nanorods are crystalline in nature. The peak was obtained at the plane (002) which corresponded to the plane at which the majority of the nanorods were present. The plane (002) indicated that the nanorods were grown standing straight up and were adjacent to each nanorod.

![Fig.21 XRD pattern for zinc oxide nanorods](image-url)
5.2 Immobilization of antibody and antigen

I analyzed the immobilization of antibody and antigen using both real time and also cyclic voltammetry (CV). I made use of the potentiostat to perform the cyclic voltammetry whose concept as explained in section 2.3.2. Cyclic voltammetry comprises of normal linear sweep voltammetry graph which is otherwise said to be a result of oxidation or reduction taking place in the device followed by the reverse sweep which measuring the re-oxidation or the re-reduction that occurs in the device.

I did the immobilization of the antibody and antigen using a cross linker called the EDC. The cyclic voltammetry curve was swept initially along the interdigitated electrodes surrounded by the standing zinc oxide nanorods. I measured the response during the presence of EDC on the top of the nanowires followed by the binding of the antibody and finally the binding of the antigen. This EDC cross linker will form a covalent bond between the antibody and the nanowires for a strong contact between the two. The EDC gets attached with the amine groups of the zinc oxide nanorods. Therefore the current increases due to the addition of EDC. This difference in current that flows across the system is realized by cyclic voltammetry curve. Fig.22 shows the cyclic voltammetry curve obtained due to the addition of EDC.

After the addition of EDC to the nanorods, the next step was to immobilize the CEA antibodies on the nanorods via a EDC layer followed by the testing of the biosensor by passing antigens over it. I did the immobilization process and the testing of the biosensor using real time measurement. As mentioned in the section 2.3.1, real time measurement was done to avoid noise interference signals and also to analyze the proper attachment of the analytes. Fig.23 shows the real time measurement of the immobilization of the antigen onto the antibody. Before the immobilization of antigens, I added the buffer solution in order to stabilize the system. The
system got stabilized and steady state measurement was done while passing over the antigens. But before the addition of the antigen onto the fully fabricated biosensor, I dropped the blocking agent named BSA so that it blocked the attachment of the target antigens onto the antibodies that were not properly immobilized.

Fig. 22 Cyclic voltammetry curve showing the presence of EDC layer
Fig. 23 Real time measurement showing the addition of BSA after stabilizing the device with buffer followed by the reaction of antigens when captured by the antibodies.

Fig. 23 represents the real time testing of the biosensor system which showed that there was only a small current change due to the addition of the blocking agent and an immediate decrease in the current due to the capturing of target antigens by the antibodies. The current response due to the binding of antigen was obtained in response to [59, 60]. When keenly observed, the current keeps on decreasing for few seconds which proves the continuous attachment of antigens to the immobilized antibodies as it was passed over the biosensor system. The reason for decrease in current was due to repelling of electrons in the nanorod in response to the negative charge present in the antibodies, which led to the formation of a depletion region in the nanorods therefore increasing the resistance of the system and decreasing its conductivity as explained in the sensing mechanism in section 4.5.6.
5.3 Cyclic voltammetry for binding of antibody and antigen

Apart from performing a real time measurement for immobilization of the antibodies and testing the system by passing the antigens, I also wanted to do IV characterization on every individual steps of building the sensor and testing it. So I performed cyclic voltammetry measurements also. The cyclic voltammetry curves shown in Fig.24 and Fig.25 were obtained after complete stabilization of each layer added to the device. I was able to see that the bare electrodes along with the zinc oxide nanowires had around some tens of micro amps current. As seen before, after the addition of the EDC layer I was able to see a large increase in current which was almost 3 milliamps. Later, when I immobilized the antibodies, decreased current resulted as seen in Fig.24 similar to the reaction obtained while performing measurements at real time was obtained. Further after I added both BSA and antigen, even more decrease in current was observed (Fig.25). This confirmed the reactions that were obtained using cyclic voltammetry was similar to the results obtained using real time.
Fig. 24 Cyclic voltammetry curve response for the binding of antibody

Fig. 25 Cyclic voltammetry curve for measurement of reaction of antigens
5.4 Immobilization of antibody with and without EDC

The main purpose of using a cross link layer (EDC) was for the covalent attachment of the antibodies to the nanowires. So in order to check the response of this interlinking layer, I performed the immobilization of the antibodies with and without the EDC layer. Fig. 26 shows the cyclic voltammetry response that was obtained while performing this test. It was observed from the figure that there was a 10X current response when the antibody was immobilized with the EDC layer in comparison to the current obtained due to the direct attachment of antibodies on the nanowires. This result proved that the attachment of antibodies occurred even without the linking layer, but a strong bond was formed between the antibodies and the nanowires due to the addition of this layer.

![Graph](image.png)

**Fig. 26** Cyclic voltammetry response for immobilization of antibody with and without EDC
5.5 Sensitivity as a function to with and without antibody

The graphs shown in the sections 5.5 and 5.6 are the most important part of the project. In this subsection I provide the proof of the concept that the sensor actually reacted only due to the capturing of the antigens on its specific antibodies and not by just addition of an extra layer to the ones that existed already. The grown zinc oxide nanorods along with the linking layer was stabilized using the buffer solution followed by the addition of blocking agent. Then antigens were directly made to flow over the linking layer and the blocking agents without antibodies. It can be seen in the Fig.27 that only about a 10% change in current occurred. This change in current might be due to the presence of the buffer solution that was present along with the target antigens. This clearly proved that the CEA antigens were not captured by bare ZnO nanorods.

![Graph showing sensitivity as a function to with and without antibody.](image)

Fig.27 Real time measurement taken by passing over antigens directly on the ZnO nanowires without immobilizing antibodies
Fig. 28 shows the graph that was plotted between percentage ratio vs. time during the attachment of antigens with and without antibody. The solid curve was obtained in response to the addition of BSA and antigen with the presence of antibody, that is, the antigens were made to directly bind onto the nanowires. The dotted curve was obtained during the attachment of antigens onto the antibodies. A 4X percentage decrease can be seen when the antigens were attached onto the antibodies in comparison to the percentage decrease seen during the antigen immobilization without antibodies. This 4X percentage decrease in terms of ratio represents the
binding occurring between the antibody and the antigen in response to the sensing mechanism explained in the section 4.5.6.

5.6 Sensitivity

The real time curve in this section is the representation of the current behavior due to various concentrations of antigens added to test the sensitivity of the device. The antigens were made to flow across the device each and every time the measurements were done. It has been already mentioned that the amount of normal blood serum level in a normal human body is 2.5ng/ml. A human can be medicated with survival possibilities if the level is below 10ng/ml. But it is a serious condition if the amount of serum level exceeds 20ng/ml. I tested the biosensor for its behavior at 10ng/ml and 20ng/ml. This was to prove that the fabricated biosensor could detect cancer at a very early stage to increase treatment options.

Fig.29 shows the comparative study that was made due to the addition of different concentrations of antigens onto the antibodies. The graph has been plotted against percentage ratio vs. time in order to show the difference in the amount of antigens that were attached. I observed that the delta percentage due to immobilization of 2µl of antigens of higher concentration (20ng/ml) was ~60% in comparison to the immobilization of 2µl of antigens of lower concentration (10ng/ml) was ~25%. The BSA and antigens were always added after 10 minutes of stabilization of the sensor using buffer solution. It was clear that higher number of antigens has been attached when antigens containing higher concentration of target cells are immobilized. Whereas when I immobilized antigens of lower concentration, the percentage of attachment was less due to fewer number of target cells present. This proved the effective
working of the biosensor which can even detect the target cells up to 10ng/ml, which when exceeded in a normal human body cannot be treated.

Fig. 29 Testing of the biosensor at two different concentrations
Chapter 6: Conclusion and Future work

In this thesis project report, I have proposed the fabrication of a biosensor for biomedical applications. I initially started the fabrication of the biosensor using polymer nanowires, particularly polypyrrole. I used the three electrode method to fabricate the nanowires with the help of an alumina template. After successful growth of nanowires, the next step was to collect the nanowires by dissolving the alumina template. As a first step, I tried dissolving the alumina template using a NaOH solution, but was not able to completely get rid of it. After several attempts by dissolving the template with higher concentration solutions I decided to use the centrifuge technique to separate the nanowires from the undissolved alumina. But I was not able to completely get rid of the template residue. So as a final step I tried to fabricate the nanowires using the template directly on the SiO2 wafer containing the deposited gold. But I faced problems of no growth of the nanowires. So I switched on to the zinc oxide nanorods for the fabrication of the biosensor.

I fabricated the zinc oxide nanorods using a solution based technique which resulted in a low cost fabrication of the device and also that the nanorods were fabricated in a standing position. This type of fabrication of nanorods resulted in growth of more nanorods at a particular surface area, which in turn helped in the improvement of the sensitivity of the biosensor as there were more nanorods. After successful growth of the zinc oxide nanowires, I started working on the immobilization of the biomolecules onto the nanowires and testing of the biosensor by passing over antigens. To test the biosensor I made use of the gold electrodes which I fabricated using lift-off process. The current that passes through the nanorods will also flow through the seed layer which is contact with the gold electrodes and thus can be viewed externally.
For the immobilization of the nanorods I made use of the covalent bonding method in which I coated a cross linking layer on the top of the nanorods before the immobilization of the antibodies. The effect of immobilizing the antibodies using a cross linking layer was proved in the results section. The nanowires along with the antibody completed the fabrication of biosensor. After the fabrication of the biosensor, I tested it by passing different concentrations of target antigens to test the sensitivity of the device. I also tested the impact of antigens without the presence of antibodies by just passing over the target antigens on the nanowires along with EDC without antibodies. The obtained results are provided in the report.

Future work is to improve the sensitivity of the sensor by increasing the length of the nanorods. I believe that by increasing the length of the nanorods, the electron transfer from the biomolecules to the seed layer will be large in number therefore increasing the response time of the device when the target antigens are made to pass through. Also there is small issue of gap between the gold electrodes and the nanorods after the fabrication of the electrodes for which the reason is unknown. I believe we can figure out the problem and fabricate the electrodes in such a way that there will be no gap in between.
References


51. Haikuo, S., Ming, L., Wenjian, W., Kui, C., Piyi, D., Ge, S., Gaorong, H., “Room-temperature preparation of ZnO nanosheets grown on Si substrates by a seed-layer assisted solution route”, Nanotechnology 19, 125603, 2008.


Appendix A: Description of Research for Popular Publication
Zinc Oxide based biosensor for the detection of lung cancer at an early stage

There are many medical devices that are commercially available in the market for various purposes for example diabetes. Previously these devices were only used in the hospital for testing. But at present there are hand held devices available by which people can detect the presence of certain disease and be aware of when it reaches an extreme stage. Cancer is one of the major diseases by which maximum of the population all over world die every year. And the serious note is that there is no cure available for disease till date. But it can be medicated when they are detected at an early stage. Keeping this as the main aim I (Anish Kumar, M) and my professor Dr. Taeksoo Ji developed a biosensor device that can detect cancer at an early stage. We have done the base level of the project by building the device using zinc oxide nanorods and testing it for various levels of concentrations of infected cells. Further we plan to make it as a device that will have a pointer that will keep on increasing as the infected cell in the blood increases and will show a red light when the presence of cancer cell exceed its normal range. We have fabricated the device using cheap methods so that the final product can be made available at a cheap price that even a common person can afford. If this biosensor is developed as a device and become commercially available, people will be able to use the presence of cancer being at home and can approach to a hospital if the level of infected cells is at an early stage.
Step by step procedure for fabrication of transducer
Appendix B: Executive Summary of Newly Created Intellectual Property

The following list of new intellectual property items were created in the course of this research project.

1. Using a solution process technique for the fabrication of zinc oxide nanowires for low cost fabrication of the device.

2. Eliminating the step of aligning of nanowires by growing the nanowires directly on the wafer along the electrodes resulting in time saving of the fabrication of the sensor
Appendix C: Potential Patent and Commercialization Aspects of listed Intellectual Property Items

C.1 Patentability of Intellectual Property

1. The solution process technique cannot be patented since the idea has been adapted from a published journal.

2. The process of growing nanowires directly on the nanowires cannot be patented since the idea has been adapted from a published journal.

C.2 Commercialization Prospects

1. No, see appendix C.1, 1.

2. No, see appendix C.1, 2.
Appendix D: Broader Impact of Research

Impact of Research Results on the Environment

- The result of this research does not have any immediate impact on the environment.
Appendix E: Microsoft Project for MS MicroEP Degree Plan
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Reading a research paper</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Literature survey for project</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Purchase of chemicals and apparatus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Purchase pyrrole, lithium perchlorate and ethyl acetate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Purchase antigens and antibodies</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Develop nanowires perfectly</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Develop gold nanowires for samples 1-4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Develop oly nanowire with silver as base for sample 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Develop oly nanowire with silver as the base</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Purchase potentiostat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----</td>
<td>-----------------------------------------------</td>
<td>------------</td>
<td>------------</td>
<td>----------</td>
<td>-----------</td>
<td>-----------</td>
<td>-------------</td>
<td>----------------</td>
<td>--------------</td>
<td>---------------</td>
</tr>
<tr>
<td>11</td>
<td>purchase polyvynyl solution</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>develop interdigitated electrodes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Develop nanowires using polyvynyl solution</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Put the antibodies and test the system</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>prepare paper for conference</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Prepare for the Journal review paper</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Collect the nanowires by dissolving the template</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>Grow nanowires using polycarbonate filters</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>Remodel the Conference paper for submission</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>Grow the nanowires with Polyvynyl solution</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ID</td>
<td>Task Name</td>
<td>October 2010</td>
<td>November 2010</td>
<td>December 2010</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----</td>
<td>----------------------------------------------------------------</td>
<td>--------------</td>
<td>---------------</td>
<td>---------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>Prepare for the class presentation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>Electroplate metal after evaporation on PC filter</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>Attend the Conference</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>Prepare the forms for the biosafety committee</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>Attend the biosafety protocol committee</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>Try growing the polypyrrole nanowires directly on gold surface</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>Learn about Potentiostat and its functions</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>Deposit nanowires on electrodes and do IV measurement</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>Prepare zinc oxide nanowires using solution method</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>Prepare for Exams and presentations</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendix F: Identification of All Software Used in Research and Thesis Generation

Computer #1:
   Model Number: Dell Inspiron
   Location: ENRC 2933
   Owner: Anish kumar

Software #1:
   Name: Electrochemical impedance spectroscopy
   Purchased by: Dr. Taeksoo Ji
Appendix G: All Publications Published, Submitted and Planned

- Anish Kumar, M., Jung, S., Ji, T., “Development of cancer biosensor based on Wheatstone bridge principle using template assisted polymer nanowires”, Accepted to IASTED International Conference on nanotechnology and Applications (NANA 2010) at Cambridge, MA (Nov 1-3, 2010).


- Anish Kumar, M., Jung, S., Ji, T., “Protein biosensors based on polymer nanowires, Carbon nanotubes and Zinc oxide Nanorods”, accepted in the SENSORS journal.