One- and two-dimensional nanostructures for chemical- and biosensing

Magnus Willander, Omer Nour and Muhammad Israr Qadir

Linköping University Post Print

N.B.: When citing this work, cite the original article.

Original Publication:

Copyright: Elsevier. Under a Creative Commons license http://www.elsevier.com/

Postprint available at: Linköping University Electronic Press http://urn.kb.se/resolve?urn=urn:nbn:se:liu:diva-93216
One- and two-dimensional nanostructures for chemical- and biosensing

M. Willander\(^{a,*}\), O. Nur\(^{a}\), M. Q. Israr\(^{a}\)

\(^{a}\)Department of Science and Technology, Campus Norrköping, Linköping University, SE- 601 74 Norrköping, Sweden

Abstract
Nanostructures have been very popular for several years to do research on sensing. In this paper we will demonstrate the advantage of using nanowires of zinc oxide (ZnO) as one dimensional structure for potentiometric measurements in biological environments. The developed procedures show suitability for the accurate determination of most of the important metal ions and to characterize cells and thin bio-layers. We will also show how to use these structures for biosensing of glucose, cholesterol, and the application of extended gate metal oxide semiconductor field effect transistors as the signal transducer. In the second part, we will show how to use a two-dimensional nanostructure, specifically graphene, for cholesterol and glucose biosensing.

© 2011 Published by Elsevier Ltd.

* Corresponding author. Tel.: +46-13-281000; fax: +46-11-363270. E-mail address: magwi@itn.liu.se.

Keywords: ZnO nanostructures; chemical sensing; biosensors

1. Introduction

A nanostructure being one dimensional (1D) or two-dimensional (2D) with relatively large surface area to volume ratio possesses attractive fundamental as well as technological properties of potential for application as sensing elements. The relatively larger surface area to volume ratio of small structures i.e. nanostructures, compared to other objects make them perform with high sensitivity when sensing small biological analytes or other metallic ions [1]. On the other hand zinc oxide as a material is characterized by features of potential for sensing (bio-safe and bio-compatible) combined with growth and synthesis possibilities that enable the design of sensitive small sensing probes [2]. The possibility to grow ZnO nanowires with sizes comparable to the size of biological analytes or other ions makes them natural electrical signal transducers. Further, the ionicity of ZnO makes it possible to functionalize the surface through electrostatic forces to tune the sensitivity of the probe. We have developed a technique that relies
on the growth of ZnO nanowires (NWs) on the tip of sub-micrometer glass pipette to produce a relatively ‘small’ selective electrodes [2]. This technique enable the accurate extra- and intracellular sensing of metallic ions and other biological analytes with non-destructive invasive penetration of biological environments being cells of thin layers [2]. We will here below present some of these recent results.

On the other hand, graphene although has been under the focus of researchers due to many fundamental findings possibilities, is of important technological applications [3]. The surface to volume ratio for 2D graphene sheets is even much higher than that of typical nanowires and hence possesses even higher surface sensitivity. This combined with the other excellent and rapid signal communication of graphene nano-sheets enforce them on the research community as new materials to develop ‘small’ and sensitive biosensors. In this extended abstract we present some of our recent findings in potentiometric sensors developed using low dimensional structures of ZnO and graphene nano-sheets. The sensing results to be shown are for sensing metallic ions and other biological analytes.

2. Experimental

The 1D sensing elements based on ZnO NWs constitute an extension to the ion sensitive electrode procedure. Using a low temperature (down to 50 °C) chemically engineered procedure can be used to grow a uniform assembly of these NWs on the tip of glass pipettes [3]. Figure 1a shows a scanning electron microscope (SEM) of typical submicrometer probe. The details of the growth procedure can be found in [4, 5]. Using such working electrode versus Ag/AgCl reference electrode different metallic ions and other biological important analytes like e.g. glucose, cholesterol etc. can all be measured very accurately. The measurement regime is based on detecting the potentiometric effect and the measurements are performed in small biological environments being cells or biological thin layers of thickness of few micrometers. To validate the measurements for cases on intracellular investigations, the technique was thoroughly investigated to insure the source of the measured signal. This is illustrated in Figure 1b-c. Here different types of working electrodes are designed. In Figure 1b the case of a working electrode with ZnO NWs sensing elements completely inserted inside the cell under measurement is shown, while in Figure 1c part of the sensing ZnO NWs are shown. To validate our intracellular measurements, we change the concentration of the ion or analyte under investigation in the buffer solution outside the cell, then using both configurations, the observed change of the electrical signal is recorded. Only in the case of Figure 1c electrical signal change is observed when the concentration is changed in the buffer solution. This confirms that the observed signal provides information from inside the cell when using this technique [2].

![Fig. 1. (a) Scanning electron microscope image showing ZnO nanowires grown on the tip of a sub-micrometer glass pipette, in (b) and (c) schematic diagrams showing the configurations used to validate the measurements.](image)
The graphene nano-sheets used in the sensing of cholesterol is shown in Figure 2a-b. In Figure 1a an atomic force microscope image (AFM) is shown, while in Figure 2b a transmission electron microscope (TEM) image is shown with the high resolution TEM as an insert showing the high crystalline quality of the graphene nano-sheet.

![Graphene nano-sheets image](image)

Fig. 2. (a) AFM image depicting a thin material of graphene nano-sheets. (b) TEM image of almost transparent graphene nano-sheet. The insert illustrates high resolution transmission electron microscope image showing good crystalline structure of graphene layer.

3. Results and discussion

For using 1D ZnO NWs for sensing we will here show some results about the intracellular evaluation of glucose inside human adipocytes cells. For this intracellular glucose sensing, Glucose oxidase solution, 5 mg/ml, was prepared in 10 mM Phosphate Buffered Saline (PBS) containing 1.5 mM Na2HPO4, 48mM KH2PO4, 0.135 mM sodium chloride and 2.7 mM KCl at pH 7.4, by using glucose oxidase (E.C. 1.1.3.4) from Aspergillus niger type GO3A, 360 U/mg (BBI Enzymes Ltd, UK). Glucose oxidase was electrostatically immobilized by dipping the tip of a borosilicate glass capillary with well aligned ZnO nanorods into 2 mL of the enzyme solution for 15 minutes at room temperature and then drying it in air for more than 20 minutes. Then the working and reference electrode were gently inserted inside the cell (as shown in Figure 3a). Human adipocytes (fat cells) were isolated by collagenase digestion of pieces of subcutaneous adipose tissue obtained during elective surgery at the university hospital in Linköping, Sweden (all patients gave their informed consent, and procedures were approved by the local ethics committee). The adipocytes were incubated overnight before use and used in a Krebs-ringer solution buffered with 20 mM HEPES, pH 7.40 and with additives. A glass slide substrate (5 cm length, 4 cm width, and 0.17 mm thickness) with sparsely distributed fat cells was placed on the pre-warmed microscope stage set at 37°C. The indicator electrode and reference electrode were mounted and micro-manipulated into the adipocytes. The measurement and evaluation of the intracellular glucose inside adipocytes cells has resulted in a value of 50±15 μM (n=5), this value is very well consistent with previous measurements using nuclear magnetic resonance spectroscopy technique. After that the insulin uptake was investigated. For that (see Figure 3b) 10 nM insulin was added to the cell medium, the glucose concentration in the cell was then increased from 50±15 to 125±15 μM it took few minutes to give the final increment for the intracellular glucose uptake.

On the other hand 2D graphene nano-sheets as those shown in Figure 2 were used to sense cholesterol. Using reference electrode versus graphene nano-sheets functionalized with cholesterol oxidase it was possible to accurately measure the cholesterol concentration over a wide dynamic range from 1 μM and up to 1 mM. Figure 4 below shows the potentiometric response of the developed graphene nano-sheet bio-sensor.
Fig. 3. (a) Human adipocytes cell under measurement of glucose concentration, and in (b) the insulin activation of glucose uptake is shown.

Figure 4 above shows the calibration curve of sensing cholesterol and it indicates a linear response over the whole measured range. This probe was used to measure the intracellular cholesterol in different types of cells. Further we have before shown that such sensors can be coupled to conventional metal oxide semiconductor field effect transistor at the gate and the sensed signal caused measurable change at the gate [6].

![Calibration curve of the electrochemical response of the graphene biosensor to cholesterol.](image)

Fig. 4. Calibration curve of the electrochemical response of the graphene biosensor to cholesterol.

4. Summary

In summary different nanostructures with 1D or 2D nature have been used for sensing of metallic ions and other important biological analytes. The procedures developed here can be very suitable for applications in relatively small biological environments, e.g. cells and thin bio-layers. The nanostructures showed high sensitivity as expected. Both used materials, i.e. ZnO and graphene nano-sheets were providing sensors with high sensitivity to the species in question. In addition the newly developed low temperature synthesis of such nanostructures will enable their integration with other soft electrode to develop smart sensors. Such nanostructures based bio-sensors are of potential for future development of physiological sensors for disease monitoring and drug delivery.

5. References