Polymer based micro sensors arrays for pH and glucose monitoring

O. Korostynska, K. Arshak, A. Arshak*, E. Gill*, P. Creedon*, S. Fitzpatrick

ECE Dept., University of Limerick, Limerick, Ireland
*Dept. of Physics, University of Limerick, Limerick, Ireland
Tel.: +353 61202267 Fax: +353 61 338176 E-mail: khalil.arshak@ul.ie

Abstract

Novel method of manufacturing micro sensors arrays for biomedical applications using BioForce NanoeNabler^{TM} is reported. The operation of pH and glucose sensing elements is based on the properties of polymers, which exhibit a change in their electrical characteristics (such as resistance or capacitance) on exposure to solutions with different concentrations of pH or glucose. A sensor for glucose was successfully fabricated using the enzyme glucose oxidase immobilized within the polymer poly (*o*-phenylenediamine). This sensor was then successfully miniaturized utilizing immobilization for a dry process. The concentrations used for the microsensor were between 1 mM and 6 mM. Samples containing different concentrations of glucose were applied to the sensor while the system was being monitored for variances in either current or conductance. The resulting changes in the electrical characteristics of the sensor monitored in real time were found to be proportional to the different concentrations of glucose applied. Microscaled interdigitated electrodes were used for sensors array, with 48 sensors places on one chip. It is envisaged that findings of this work would form the basis for miniaturised point-of-care diagnostic system.

Keywords: Microsensors, nanopatterning, real-time monitoring, pH, glucose, interdigitated electrodes

1. Introduction

The value of pH has significant kinetic and thermodynamic effects on biochemical reactions, since the apparent free energy of a reaction depends on the composition of the reaction medium (H^+, ionic strength, and metal ions that have significant binding constants) and its temperature [1]. There is a need for accurate and instant measurement of pH values in a wide range of applications, such as environmental monitoring (water quality), blood pH measurements and laboratory pH measurements amongst others [2, 3]. Intracellular pH is an important modulator of cell function. The activity of most proteins is affected by even small changes of proton concentration, and a number of cellular mechanisms exist that finely regulate the intracellular pH (pH_i) value [4]. Consequently, monitoring pH_i with high spatial resolution can help elucidate many physiological or pathogenic processes [5].

The most common systems for pH sensing are based upon either amperometric or potentiometric devices. Ion selective membranes, ion-selective field effect transistors, two terminal microsensors, fibre optic and fluorescent sensor, metal oxide and conductometric pH-sensing devices have also been developed [6, 7]. However, these types of devices can often suffer from instability or drift and, therefore, require constant re-calibration.

The research on miniaturized polymer based pH sensors has recently emerged due to the progress made in polymer materials science [8]. By introduction of functional groups, polymers can be designed to selectively swell and shrink, thereby changing mass and elasticity, as a function of analyte concentration. The ion-exchange properties of conducting polymers are of special interest for potentiometric sensor development [9, 10, 11]. Conducting polymers are ideally suited for sensor applications because they not only exhibit high conductivity and electroactivity but they could also be used as a general matrix and can

be further modified with other compounds in order to change selectivity [12]. Compared to conductive polymers, nonconductive ones usually have high selective response and high impedance, which is important for eliminating interference by other electroactive species [13].

The pH sensing properties of polyaniline (PANI), which belongs to the group of organic conducting polymers, were explored by Tsai et al [14]. The use of PANI receives attention due to its high conductivity, ease of synthesis, and its stability under ambient conditions. Fig. 1 depicts the spectral response in the pH range from 2.15 to 12.54 of PANI film deposited onto ITO glass employing constant-potential (0.80 V) electropolymerization [14]. These pH-dependent spectral variations of PANI films were explained by their transformation from the protonated to the unprotonated form.

It has been shown that electromodified electrodes with conducting polymers could act as good candidates for replacement of popular glass pH electrodes [13]. The examples of pH chemical sensors using polymer-film-coated electrodes include electropolymerization of pyrrole, aniline, thiophene, or benzene derivatives [13, 15, 16, 17, 18]. However, the measurements of pH using the above-mentioned conductive polymers had poor reliability due to defects and pinholes present in the films structure. Herlem et al [13] reported a pH sensor, where a smooth Pt electrode was coated with an electrically insulating polymer, namely linear polyethylenimine (L-PEI). Platinum electrodes, modified by a coating of a thin L-PEI film, resulting from the anodic oxidation of pure ethylenediamine, exhibited a linear, reversible, and stable in time potential response sensitive to pH changes in aqueous media. The assembly of the electrode surface coated with electropolymerized ethylenediamine acted as a transducer of the electrode potential versus the pH value in aqueous solutions. A possible mechanism by which the linear polyethylenimine responds to pH changes could be due to the affinity of the numerous amino groups to the protons in solution [8].

Herlem et al [13] reported that as the film thickness increases, the more amino groups are present, and thus the pH sensitivity of the modified electrode increases. These thick film modified electrodes give repeatable pH responses, independent of direction of pH change, and give consistent results when the pH value is varied in a random manner.

Diabetes is a worldwide public health problem. This metabolic disorder results from insulin deficiency and hyperglycemia and is reflected by blood glucose concentrations outside the normal range of 80-120 mg/dL. The complications of battling diabetes are numerous. The diagnosis and management of diabetes requires a tight monitoring of blood glucose levels. Accordingly, millions of diabetics test their blood glucose levels daily, making glucose the most commonly tested analyte. Amperometric biosensors based on oxidase enzymes are the most widely used biosensors. Through a catalytic enzymatic reaction between glucose and glucose oxidase the biosensor generates hydrogen peroxide and its response therefore is based on the electrochemical oxidation of H_2O_2 [9]. The electrochemical formation of polymer films enables to make a glucose sensor with a controlled thickness, which is reproducible and miniaturized [19]. Nonconducting polymers such as poly(*o*-phenylenediamine) (PPD) films, which exhibit good selectivity, have been successfully utilized in this regard [20, 21, 22].

Various biosensors and microarrays appeared in the late 1980's as a major technological breakthrough, not the least due to the advanced use of photolithography process applied to the implementation of biochips and progress in surface chemistry, biology, microfluidics, instrumentation, electronics, optics and bio-informatics [23, 24]. Technologies that are employed at each step should be versatile enough to adapt to different challenges and needs, depending on the application in mind. The research towards integration of multi-sensor head for simultaneous in vivo monitoring is ongoing. This papers reports on the development of a single chip for the detection of both pH and glucose levels using microsensor arrays based on interdigitated electrodes.

2. Experimental procedure

The process for the manufacture of microscaled interdigitated electrodes for sensors array was as follow. A p-type silicon wafer was utilized, on top of which 1 μm silicon oxide layer was thermally grown using a Thermco 9000 furnace. Then a bi-level resist system with Micro-chem LOR3A and Shipley S1813 was spun on, exposed and developed in a bath of Microposit MF319 developer. After undeveloped resist was rinsed off, a deposition of 200 nm gold using a Temescal FC-2000 E-beam evaporator was performed. Afterwards, the photoresist was etched in a bath of Microposit 1165 resist-stripper, which resulted in lifting-off the gold on the photoresist, but leaving the gold electrodes on the oxide. Fig. 1a) illustrates Scanning Electron Microscope (SEM) image of the resultant electrode arrays, whereas Fig. 1b) depicts the interdigitated electrode structure of an individual sensor within the array. The width of each electrode is 5 μm.

a) b)

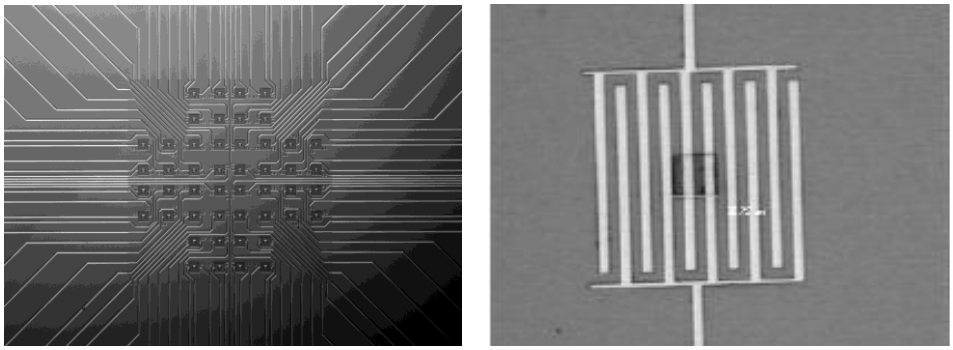

Fig. 1. a) SEM image of the sensors array chip with 48 interdigitated electrodes, b) interdigitated electrode structure of an individual sensor.

Novel nanopatterning technology offered by BioForce NanoeNabler^{TM} was successfully tested for developing various sensors, with the focus on nano sensors arrays. This system uses a liquid dispensing process via specially designed surface patterning tool (SPT), which is microfabricated cantilever with an integrated passive microfluidic system. Fluid loaded into the reservoir flows down the microchannel by capillary flow until it reaches the gap at the end of the SPT. During the deposition process, which typically takes less than 100 msec, SPT end touches the surface and a volume of fluid is instantly transferred. The Nano*e*Nabler^{TM} can deliver attoliter to picoliter volumes of liquid with a high degree of spatial accuracy. It should be noted that a number of different polymers can be used in the form of sensor arrays, with each array element having unique selectivity and sensitivity properties. The strategy of simultaneous measurement of a number of sensor arrays relies on the application of pattern recognition technique.

3. Results and discussion

An important determinant is that the pH_i fall usually correlates with increased lactate concentration as blood glucose is increased. Elevating blood glucose to 20 mM or above enhances damage at the same time as it accentuates the rise in lactate and the fall in pH [25, 26]. In general, the rapid falls in pHi during ischemia in normoglycemic conditions are enhanced by 0.3-0.6 units at 20 mM glucose. A wireless, remote query combined glucose/pH biosensor using a ribbonlike, mass-sensitive magnetoelastic sensor as the transducer was developed [27]. The glucose biosensor was fabricated by first coating the magnetoelastic sensor with a pH-sensitive polymer and upon it a layer of glucose oxidase (GO_x) was placed. The pH-responsive polymer swells or shrinks, thereby changing mass, respectively, in response to increasing or decreasing pH values. The dissociation of gluconic acid produces H^+, which diffuses to both the bulk solution and the pH-sensitive polymer resulting in

polymer shrinking [3]. The pH-sensitive polymer is a slightly crosslinked polyelectrolyte gel synthesized from acrylic acid and isooctyl acrylate monomers. Isooctyl acrylate was used to increase the hydrophobicity so that it is undissolvable in water. In addition, the branch alkyl group seems to enhance the ability of the polymer to swell [27]. There are several arguments for biosensors to be fabricated on a microscale. Miniaturization of the sensor offers advantages over conventional medical techniques. An extremely high spatial resolution may be provided through the measurement of small features, an option which microsensors offer.

The operation of the sensing elements is based on the properties of polymers, which exhibit a change in their electrical characteristics (such as conductivity, potential or capacitance), on exposure to solutions with different concentrations of pH value [8, 3, 28, 10]. Figure 2 presents optical image of a sensor array, manufactured using different pH-sensitive solutions based on conducting polymer compositions of PANI, PS3 and Polyvinyl Butyral (PVB). The properties of the materials, as well as humidity, surface properties and other parameters would affect the final dimension of each sensor head. Once the proper parameters are chosen, the process of microsensors arrays manufacture is fast, reliable and highly consistent.

Glucose oxidase (500 units/mL) immobilized in PPD (5 mmol/L) was also deposited on the interdigitated electrodes. The arrays were then placed in an oven to dry for 1 hour at 30 °C this formed a sensor head on each electrode. A sample containing a known concentration of glucose was applied to the sensor head, with the procedure repeated again for different concentrations of glucose and utilizing several sensor heads, each being used for just one sample. HP 4277A LCZ meter was used to measure the response of each sensor to glucose addition by monitoring changes in the conductance of the sensor as a result of the enzymatic reaction. Fig. 3 shows the change in the values of conductance as a result of glucose addition with glucose concentrations ranging from 0.001 M to 0.006 M.

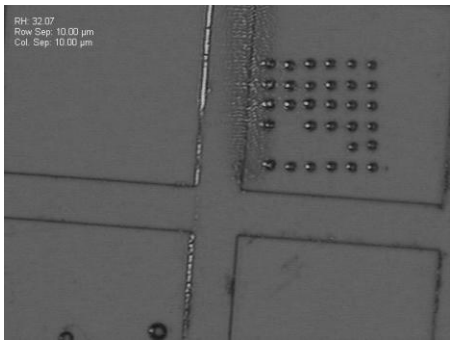

Fig. 2. Polymer pH sensors measuring 10 µm in diameter.

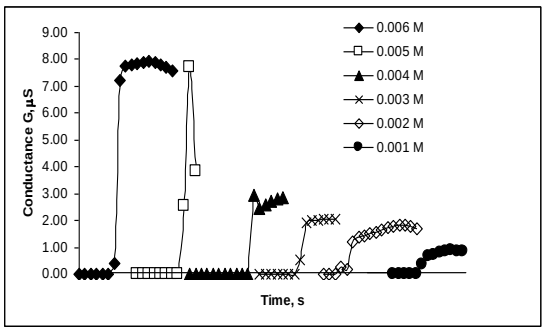

Fig. 3. Change in the values of conductance as a result of glucose addition to the microsensor.

4. Conclusion

A microscale glucose sensor and pH sensors for biomedical applications were developed using novel nanopatterning technology offered by BioForce NanoeNabler^{TM}**.** The presented methodology for the fabrication of microsensors arrays may be extended to surface patterning of a broad spectrum of various nanoscale materials and thereby create opportunities in a variety of fields ranging from microelectronics to bio/nanotechnology. It is strongly believed that the size reduction of the sensor to a few microns described in this paper creates new opportunities in the areas of chemical and biological sensor development.

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