Available online at www.sciencedirect.com
SCIENCE DIRECT®

Sensors and Actuators B 110 (2005) 382–389

SENSORS AND ACTUATORS B CHEMICAL

www.elsevier.com/locate/snb

Fabrication of polypyrrole–glucose oxidase biosensor based on multilayered interdigitated ultramicroelectrode array with containing trenches

Mingzhi Zhu [a,b,c,*], Zhuangde Jiang [a,b], Weixuan Jing [a,b]

[a] State Key Laboratory for Manufacturing Systems Engineering, Xi'an 710049, Shaanxi, PR China
[b] Institute of Precision Engineering, Xi'an Jiaotong University, Xi'an 710049, Shaanxi, PR China
[c] Institute of Structural Mechanics, China Academy of Engineering Physics, Mianyang 621900, Sichuan, PR China

Received 11 November 2004; received in revised form 5 January 2005; accepted 6 February 2005
Available online 8 March 2005

Abstract

A miniature glucose biosensor has been developed based on electropolymerization of polypyrrole–glucose oxidase on a multilayered gold interdigitated ultramicroelectrode array with containing trenches. The basal band ultramicroelectrode with a functional width of 362 nm is fabricated using multilayered materials and conventional photolithographic techniques. The electrode surface is inside the containing trenches, the depth of which is larger than 1.5 μm. High quality electrodes with uniform geometries are characterized by microscopy and electrochemical techniques. The corrosion resistance is investigated on exposure to the normal saline, which indicates that the electrodes are adequate for acute experiments lasting a few hours. Fabricated by electropolymerization, the glucose oxidase/polypyrrole (GOx/PPy) biosensors can be used for detecting glucose concentration in the linear range of 0–10 mmol/L, with a sensitivity of 13.4 nA/(mmol L) and a correlation coefficient of 0.998, respectively.

Keywords: Multilayered interdigitated ultramicroelectrode array; Containing trench; Glucose; Electropolymerization

1. Introduction

In recent years, miniaturization can be regarded as an aspect of the development of electrochemical sensors. There is a clear trend to handle small amount of analyte and a demand on constructing micro-Total Analytical Systems (μTAS) that include various functions such as the mixing, the heating and the in situ detection [1–4]. In the development of electrochemical sensors, single ultramicroelectrodes (UMEs) and ultramicroelectrode arrays exhibit a wide possible field of application ranging from medical diagnosis, biotechnology and biological research, over food control to environmental monitoring. It is difficult to manufacture single ultramicroelectrodes and ultramicroelectrode arrays with uniform characteristics. To overcome these problems, semiconductor and micromachining techniques have been employed to produce a variety of microelectrodes. These technologies are powerful tools for the construction of miniaturized structures and have gained increasing importance due to exciting and extraordinary development in the field of sensors and actuators. The advantages brought by these technologies are: reduced size, small sample volume, reliable, inexpensive, and identical, highly uniform, and geometrically well-defined element [5–7].

In this work, a submicrometer multilayered interdigitated ultramicroelectrode array (IDA) was fabricated by photolithographic patterning of multilayered materials on a quartz substrate and the band electrode surface is inside the containing trenches. Ultramicroelectrodes arrays have widened the area of applications where voltammetric and amperometric electrodes are being employed [8]. Among

* Corresponding author. Tel.: +86 292668616150; fax: +86 292668612.
E-mail address: zhumingzhi@mailst.xjtu.edu.cn (M. Zhu).

0925-4005/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.snb.2005.02.036

various geometries of UMEs arrays, the IDA appears to be of the greatest interest for electrochemical sensors where detector device and/or sample volume need to be miniaturized while achieving high current amplifications and sustaining their improved signal-to-noise ratio [2,10–13]. The technique of patterning of multilayered materials is one of the effective approaches for fabrication of submicrometer and nanometer sized band electrodes by conventional photolithographic technique, and it is a useful way of increasing the functionality per unit area of substrate by multiplying edge band electrodes along the wall of a single microfabricated feature [9,14,15]. The containing trenches of the fabricated multilayered IDA may provide adequate membrane adhesion and sufficient mechanical stability for the sensing membranes immobilized in the trenches [16–19]. Electrochemical behavior of the multilayered IDA with containing trenches have been obtained using cyclic voltammetry (CV) and the size and physical features have been characterized by means of optical microcopy, scanning electron microscopy (SEM), atomic force microscopy (AFM), and energy-dispersive X-ray analysis (EDX).

For multilayered IDA, silicon nitride thin films (PECVD) were used as the passivation materials. The passivation materials of semiconductor and micromachining techniques generally had been developed for insulation rather than corrosion protection purposes. Therefore, the serve life of the passivation materials was investigated in normal saline and surface characterization before and after corrosion testing was performed using SEM. It shows that the IDA can be used as disposable basal ultramicroelectrodes of biologic sensors [20–24]. Biosensors based on the UMEs are an attractive technique for the clinical diagnosis and monitoring devices, both in vivo and in vitro measurements. Since enzyme can be successfully immobilized in polymers through the one-step electropolymerization allowing the functionalization of complex transducer shape from macro to nanoscale, the glucose oxidase (GOx) was entrapped in polypyrrole (PPy) membrane by the electropolymerization of pyrrole monomer with the band electrode that is inside the trenches of multilayered IDA [25–31].

2. Experimental

2.1. Reagents and apparatus

Quartz substrates were purchased from Corning; and gold target and titanium–tungsten target were purchased from AT&M. Glucose oxidase was purchased from Sigma and pyrrole from SCRC. All other chemicals used in this study were analytical grade. Pyrrole was distilled prior to use. The glucose stock solution was left for about 24 h for mutarotation before use. All solutions were prepared with double distilled water.

Electrochemical characterizations were performed on a BAS 100 electrochemical analyzer (Bioanalytical Systems, Inc.) with a saturated calomel reference electrode (SCE). Electropolymerizations were done using a computerized multifunction electrochemical analysis instrument (MEC-12B, Jiangsu Electroanalytical Instrument Factory) with an Ag reference electrode. An optical microscope (Guiyang Xintian Oetech Co., JX6920021) and a scanning electron microscope (SEM) (JEOL, JSM6460, equipped with energy-dispersive X-ray) were used for visual inspection and measurements of the multilayered IDA with containing trenches. Energy-dispersive X-ray was performed for thin films elemental analysis. An atomic force microscope (AFM) (Thermomicroscopes-Veeco, Autoprobe) was employed for the measurement of thin films thickness and surface topography.

2.2. Electrode design

The design of the multilayered IDA with containing trenches used in this study is illustrated schematically in Fig. 1. The overall size of IDA is about 1.5 mm × 2.4 mm. It contains 50 pairs of gold band ultramicroelectrodes. In Fig. 1, w_f, w_g and b are the width, the intervening space and the length of the individual digit, respectively, which are equal to 11 μm, 12 μm and 1.5 mm, respectively. The 50 μm-wide conducting tracks of bond pads are located at the flanks of IDA. The contact pads are 1 mm × 1mm. The surface of the

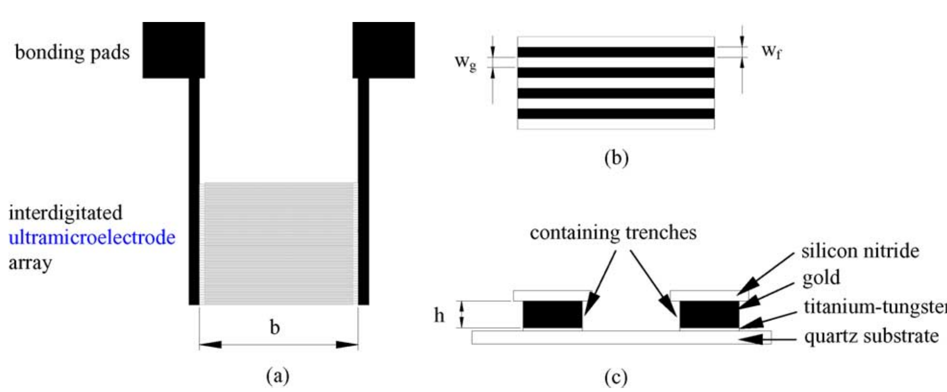

Fig. 1. Schematic diagrams of multilayered interdigitated ultramicroelectrode array with containing trenches. (a) Macroscopic configuration. b is the finger length. (b) Microscopic configuration. w_f is the passivation layer width, w_g is the interelectrode gap. (c) Microscopic cross-section configuration. h is the width of electroactive area (not to scale).