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Nanoband array electrode as a platform for high sensitivity enzyme-based glucose biosensing

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ABSTRACT

We describe a novel glucose biosensor based on a nanoband array electrode design, manufactured using standard semiconductor processing techniques, and bio-modified with glucose oxidase immobilized at the nanoband electrode surface. The nanoband array architecture allows for efficient diffusion of glucose and oxygen to the electrode, resulting in a thousand-fold improvement in sensitivity and wide linear range compared to a conventional electrode. The electrode constitutes a robust and manufacturable sensing platform.

1. Introduction

Enzyme-based electrochemical biosensors are used for a multitude of applications; they employ a wide variety of oxidoreductases, and are found in many analytical instruments used in environmental, food, pharmaceutical and clinical laboratories [1]. The stand-out, most successful application is electrochemical biosensors for glucose sensing; these now help millions of diabetic people manage their condition, and many new biosensor designs are reported yearly in the literature [2,3]. Some type 1 diabetics find it difficult to manage hypoglycaemic events, which can lead to the need for emergency medical intervention. One means to reduce the need for such intervention is to employ Continuous Glucose Monitoring (CGM) devices which are implanted under the skin, where they reside for typically a week. CGM has proven very useful for understanding and managing individual patient therapeutic regimes. However, a number of challenges are faced in this field including poor device accuracy, narrow linear range and the short lifetime of implanted devices [4]. Apart from invasive sensors, wearable electrochemical sensors have received considerable recent interest and could potentially be employed for CGM, operating in tears, sweat or saliva [5].

Many glucose biosensors use additional electroactive mediators to operate. Due to the possible toxicity of the mediators [6], their use in implantable devices is however problematic, e.g. in CGM devices. Another common way to produce a glucose sensor, also employed in the majority of the CGM devices currently on the market [4], is to use a so-called first generation glucose biosensor design, where GOx turns over glucose in the presence of its natural co-substrate oxygen, producing hydrogen peroxide [2]. Hydrogen peroxide is then electrochemically oxidised at a Pt electrode by applying an anodic potential:

$$\text{glucose} + O_2 \xrightarrow{\text{GOx}} \text{gluconic acid} + H_2O_2 \quad (1)$$

$$H_2O_2 \rightarrow O_2 + 2H^+ + 2e^-. \quad (2)$$

One drawback with such a biosensor design is that the concentration of dissolved oxygen is typically much lower than that of the glucose, meaning that the response of the device is determined by the availability of oxygen [3].Oxygen and glucose concentrations need to be controlled with diffusion membranes, which necessarily also reduces the glucose concentration at the electrode surface and generates mass transfer limitations [7,8].

By employing different nanomaterials in the design of biosensors, considerable improvements in sensitivity, selectivity, and accuracy can been achieved [9,10]. Different nanomaterials such as nanoparticles, nanofibres, nanotubes and nanocomposites have been used to modify macro-scale electrodes. Many different strategies have been developed to immobilise enzymes on these nanostructured macro-electrodes, which have resulted in enhanced redox currents. This has been achieved by stabilising surface interactions with the adsorbed enzymes and

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presenting a high surface-to-volume ratio to enable a high enzyme load [11].

Instead of employing nanomaterials-modified macro-scale electrodes in the biosensor design, another approach is to use nanoelectrodes or nanoelectrode arrays [6,12,13]. The electrochemical properties of nanoelectrodes differ significantly from those of macro-scale electrodes, and are characterised by enhanced and often steady-state (time independent) diffusion. This enhanced mass transport provides several benefits, including increased signal-to-noise ratio and high sensitivity. This has already been shown to provide high sensitivity for biorecognition reactions [13,14]. Thus, an important potential application of nanoelectrodes is in bioanalytical measurement systems, e.g. for healthcare applications, where the improved sensitivity can expand the range of target analyte molecules to include those which are currently considered to be at impracticably low levels in situ, as well as to improve the overall performance of the biodevice.

In the present paper we employ a nanoband array electrode, fabricated using processes standard to the semiconductor industry with very high reproducibility, to produce a high-sensitivity enzymatic glucose sensor operating by immobilising the glucose oxidase (GOx) enzyme on the electrode surface. This utilizes the common first-generation approach as employed in many commercial CGM devices to illustrate the potential benefit a nanoband array could have in biosensing [4]. The nanoband array electrode demonstrates a thousand-fold improvement compared to a similarly designed macro electrode, attributed to the particular properties of the nanoscale electrode architecture.

2. Experimental

2.1. Materials

All chemicals used were of analytical grade and were purchased from Sigma Aldrich, UK. Glucose oxidase from *Aspergillus niger*, 149.8 U mg^{-1}, was used for the enzyme modification. All solutions were prepared using deionised water with a measured ionic resistivity of 18.2 MΩ cm. An air-saturated 10 mmol dm^{-3} phosphate buffered saline (PBS), pH 7.4, containing 0.0027 mol dm^{-3} potassium chloride and 0.137 mol dm^{-3} sodium chloride was used throughout all experiments. Glucose stock solutions (0.1 mol dm^{-3}) were prepared with PBS and were allowed to mutarotate overnight before use. A Pt Microsquare Nanoband Edge Electrode (MNEE, Fig. 1 c) (CAVIARE™ Nanoband Array, Platinum 303D, NanoFlex Limited, UK), discussed in more detail in Section 3.1, or a 100 μm diameter Pt disc electrode were biomodified and used as working electrodes.

The fabrication processes used to obtain MNEE architectures are standard to the semiconductor industry, employing thin film techniques and lithography. As a result the device yields are extremely high (>98%) with excellent reproducibility from device to device (typical relative standard deviations < 1%). The approach is also compatible for use with flexible substrates using processing techniques that are currently widely used for the mass production of commercial disposable electrochemical test strip devices at comparable manufacturing costs.

2.2. Electrode modification

Prior to use, the MNEE was cleaned electrochemically by cycling the potential between −0.35 and 1.9 V (vs. SCE) at a scan rate of 100 mV s^{-1} for 10 cycles in 0.05 mol dm^{-3} H_2SO_4. The MNEE was then incubated in conc. H_2SO_4 for 3 min followed by 50% H_2SO_4 for another 3 min and thereafter rinsed copiously with water. To create the glucose sensor, the Pt MNEE was first modified with 6-Mercaptohexylamine thiol (MHA). Apart from gold, thiols are also known to form well-defined monolayers on other metals, such as Pt [21,22] Thiol modification was performed by incubating the MNEE for 20 h in 50 μmol dm^{-3} of MHA dissolved in ethanol. The electrode was thereafter rinsed with ethanol followed by water. After thiol modification, the electrode was incubated for 45 min in 20 μL 8% glutaraldehyde solution (dissolved in water). Afterwards, the electrode was carefully rinsed with buffer and 20 μL of 40 mg ml^{-1} GOx (dissolved in buffer) was pipetted over the MNEE and left covered to prevent evaporation for 2 h. The electrodes were finally rinsed with buffer and ready for use. The Pt disc electrode was prepared in a similar.

2.3. Electrochemical measurements

All electrochemical experiments were carried out in a Faraday cage using a DY2322 digital bipotentiostat (Digi-IVY, USA) The electrochemical cell consisted of a three-electrode system using a Pt MNEE or Pt disc electrode as working electrode, platinum wire counter electrode and a saturated calomel electrode (SCE) as reference. All electrochemical experiments were carried out at room temperature. Prior to addition of analyte, the working electrode was pre-conditioned by cycling the potential 10 times between 0.2 and 0.9 V, where hydrogen peroxide will be oxidised at the platinum surface. For glucose measurements, the measurement solution was stirred at 600 rpm using a magnetic stirrer bar to prevent build-up of hydrogen peroxide. Calibration curves of enzyme modified electrodes towards glucose were constructed from chronoamperometry measurements at +0.7 V vs. SCE, averaging the current at 60 s over 3 repeats, whilst stirring the solution.

3. Results and discussion

3.1. Glucose sensing at MNEE vs. disc electrode

We have previously demonstrated a Platinum (Pt) Microsquare Nanoband Edge Electrode (MNEE) structure which was designed and produced by utilising standard semiconductor processes and

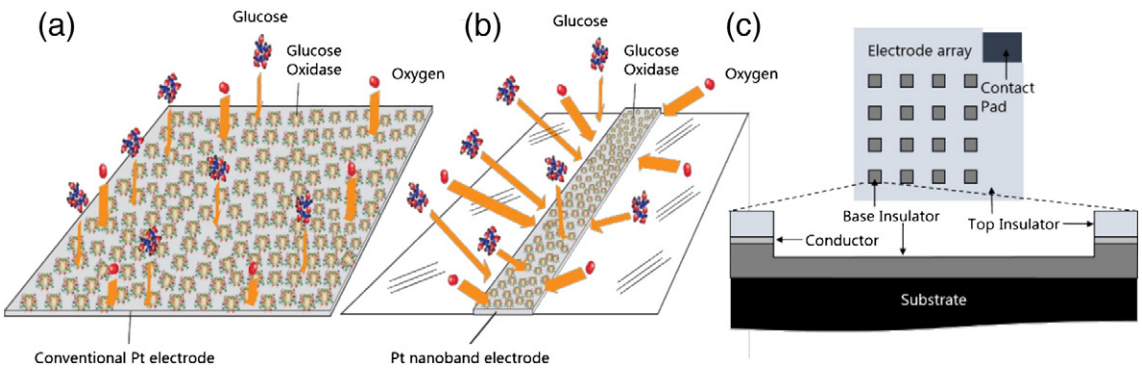

Fig. 1. Diffusion field at a conventional and nanoband electrode. Illustration of the planar diffusion field of a conventional planar electrode (a) compared with the local diffusion field in close proximity to a single nanoband within an aperture (b). (c) Schematic of the MNEE, not to scale.