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Design and development of novel screen-printed microelectrode and microbiosensor arrays fabricated using ultrafast pulsed laser ablation

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ABSTRACT

A new generic platform for the development of microbiosensors combining screen-printing and ultrafast pulsed laser technologies has been developed, characterised and evaluated. This new platform consists of a layer of screen-printed carbon ink containing the enzyme and mediator, covered with an insulating layer formed from a dielectric screen printed ink. Microholes were drilled through the insulated layer by ultrafast pulsed laser ablation to generate the microbiosensor array. The geometry of the microelectrode array was evaluated by optical microscopy, white light surface profiling and scanning electron microscopy. The electrochemical behaviour of the microelectrode array was characterised by cyclic voltammetry and compared with macroelectrodes. The analytical performance of the microbiosensor array was evaluated with external counter and reference electrodes for hydrogen peroxide and glucose determination showing linearity up to 4 mmol L^{-1} and 20 mmol L^{-1} (360 mg dL^{-1}) respectively. The full screen printed three-electrode configuration shows linearity for glucose determination up to 20 mmol L^{-1} (360 mg dL^{-1}). This study provides a new fabrication method for microelectrode and microbiosensor arrays capable for the first time to retain the activity of the enzymatic system after processing by pulse laser ablation.

1. Introduction

The increasing interest in the production of microelectrodes is due to factors such as fast response times, high signal-to-background noise ratios, the ability to operate in low conductivity media, and the generation of steady state responses [1]. Microelectrodes have been fabricated using a variety of methods to produce different designs [2–4]. Our group has investigated the construction of microband devices which have been fabricated by covering the screen-printed carbon electrode (SPCE) working area with polyvinyl chloride (PVC) insulation tape, then cutting transversely across the centre of the working area [5,6]. These microband biosensors were subsequently used to monitor changes in glucose concentration in cell culture using the HepG2 (human Caucasian hepatocyte carcinoma) liver cell line [7]. In another approach we have produced tubular microband electrodes by drilling a hole through the two conventional electrode surfaces and polyester substrate covered with a dielectric layer [8]. Other groups focus on the development of disposable microelectrodes completely by screen-printing technique [9,10]. Also, microelectrodes have been manufactured by combining microphotolithography and screen-printing technology to produce microbiosensors capable of monitoring glucose metabolism in 96-well format cell cultures [11]. The majority of approaches to microelectrode fabrication are based on gold electrodes and conventional fabrication techniques such as photolithography with the main disadvantage of production cost [12–16].

Recently, microelectrode fabrication using femtosecond laser has been successfully demonstrated to have significant advantages over conventional (photolithographic) fabrication methods, in that it provides a fast and flexible approach to microelectrode generation [17,18]. Laser etching systems have been used to create holes in polyester sheets with the aim of creating microelectrode behaviour for electrochemical paper-based analytical devices (ePADs) [19]. Therefore, in the present study we wished to explore the possibility of developing a generic platform for the mass production of biosensors using the convenient combination of screen-printing

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and pulse laser technologies. The screen-printing process is particularly suitable for the production of low-cost disposable biosensor devices [20,21] and has been employed in the manufacture of the test strips commonly used by diabetic people to monitor their blood glucose levels [22]. Such biosensors are usually fabricated using a screen-printing carbon ink that usually contains redox mediator [23].

In previous studies, we have demonstrated the possibility of incorporating enzymes and mediators directly into a screen printing ink for the mass production of amperometric biosensors [24,25]. We considered that a combination of screen printing and pulse laser technologies could lead to a new generic platform for the development of microbiosensors. We envisaged that the basic platform for the fabrication of microbiosensors would consist of a layer of screen-printed carbon ink containing the enzyme and mediator, covered with an insulating layer formed from a dielectric screen printed ink. The intention was to use pulse laser ablation to drill microholes of appropriate size through the insulated layer to generate the microbiosensor array. One of our main goals was to produce a microelectrode array, and microbiosensor array, that would generate steady state currents in unstirred solution; the latter array has an important application in monitoring cell metabolism and in toxicity studies.

This paper is divided into two main parts. The first part describes the fabrication of a microelectrode array based on the combination of screen-printing and pulse laser ablation using ferricyanide as a redox probe. In the second part, we describe the development of a glucose microbiosensor array based on the previous optimised microelectrode geometry. It should be emphasised, that mass production of such devices could readily be feasible using the approach described in this paper.

2. Materials and methods

2.1. Instrumentation

The electrodes were printed using a DEK 1202 screen printer. Laser processing by a femtosecond laser (Clark MXR CPA 2010, 150 fs, 775 nm, 1 kHz) and picosecond laser (HighQ IC-355-800, 10 ps, 1064 nm, 50 kHz) was carried out in the Lairdside Laser Engineering Centre, Liverpool. Visual evaluation of the microelectrode surface was carried out by Digital Blue microscope model QX5 with magnifications ranged from 10× to 200×. The Scanning Electron Microscopy (SEM) images were taken with Phenom SEM microscope (FEI Company). White light surface profiling was obtained with WYKO NT1100 optical profiling system. An Autolab PSTAT 10 computer-controlled potentiostat (Windsor Scientific, Slough, UK) was used for all electrochemical studies. A three-electrode system comprising a platinum counter electrode, a double junction Ag/AgCl reference electrode with 0.1 mol L^{-1} KCl as the external reference solution and the microelectrode working electrode was used in all experiments conducted in three-electrodes configuration.

2.2. Chemicals and reagents

All chemicals were of analytical reagent grade and obtained from Sigma–Aldrich (Gillingham, Dorset, UK). The supporting electrolyte used throughout was phosphate buffer 0.1 mol L^{-1}, KCl 0.1 mol L^{-1} prepared in deionised water by mixing solutions of KH_2PO_4 and K_2HPO_4 to obtain the desired pH 7.5 (PB). In the cases needed, 0.5 mmol L^{-1} of ferricyanide was used as mediator. The stock solution of 0.5 mol L^{-1} hydrogen peroxide was made up fresh in PB. Standard solutions of β-D-glucose were prepared in the appropriate volume of PB and allowed to mutarotate at room temperature.

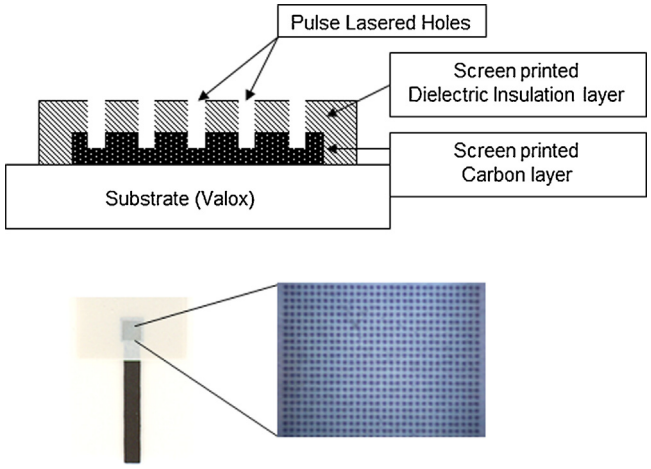

Fig. 1. Schematic illustration of the print layers and the lasered holes (up) and photograph of the screen-printed microelectrode (down).

Carbon (C10903D14, C2030519P4, C2030408P3 and C2070424D5) and dielectric (D2080603D3, D2050823D13, D2070423D5, D2060131D1, D2000222D2 and D50706D2) ink formulations from Gwent Group Ltd. were used for the optimisation of the microelectrode geometry, mediated system evaluation and glucose biosensors development.

2.3. Microelectrode fabrication and optimisation of the geometry of the array

Microelectrodes were fabricated on a valox base by screen printing a commercially available carbon ink formulation, C10903D14, giving a working area of 3 × 3 mm. The carbon layer was covered with a screen printed dielectric before processing by laser to create microholes of a given size and spacing over the working area (Fig. 1).

The coverage of the dielectric print layer was evaluated visually under the microscope for different dielectric inks based on polyester (D2080603D3, D2050823D13 and D2070423D5) or acrylic (D2060131D1, D2000222D2 and D50706D2) resins.

Parameters for the laser processing were optimised to produce the correct hole definition and depth. The pulse power energy and scan rate number were adjusted to give the right penetration through the dielectric to expose the carbon layer without penetrating through the substrate. One set of electrodes were processed using the femtosecond laser (150 fs pulsed laser radiation) at a wavelength of 775 nm and another set using the picosecond laser (10 ps pulsed laser radiation) at a wavelength of 1064 nm. In order to optimise the geometry of the microarray different diameter holes ranged from 20 to 60 μm at different distances apart ranged from 30 to 540 μm were processed. White light surface profiling and Scanning Electron Microscopy were used to visually assess the surface of the exposed carbon and the hole size and profile. The electrodes were observed without any pre-treatment. After fabrication, the microelectrodes were examined electrochemically as described in Section 2.5.1.

2.4. Design of glucose microbiosensor

The possibility of developing a glucose microbiosensor was explored using pulsed laser ablation to create microholes in a dielectric which covered the underlying enzyme layer. Fig. 2A shows the reactions involved in the glucose microbiosensor under evaluation. The base transducer consisted of a microelectrode array produced in the manner described in Section 2.3 using a water