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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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HIGHLIGHTS

- A new method for fabricating microelectrode and microbiosensor arrays is proposed.
- The generic platform combines screen-printing and ultrafast pulsed laser technologies.
- The activity of the enzymatic system was retained after processing by pulse laser ablation.
- Steady-state responses were obtained using chronoamperometry.
- A prototype glucose microbiosensor array was developed.

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.

Keywords: Microelectrode; microbiosensor; laser ablation; screen-printing; microarray; enzymatic biosensor

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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 consequently 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 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

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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 10X to 200X. The Scanning Electron Microscopy (SEM) images were taken with Phenom SEM microscope (FEI Company). White light surface profiling was obtained

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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.

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 x 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 (Figure 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.

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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. Figure 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 based carbon ink containing cobalt phthalocyanine (CoPC) as mediator (C2030408P3). No enzyme was incorporated at this stage (Figure 2B). Next, microbiosensors were produced as described above using a water based carbon ink containing CoPC as mediator and glucose oxidase (GOX) (C2070424D5) reported in previous work by Crouch *et al* [24, 25]. Finally, these were printed in a three-electrode configuration (BE2090701D1, Figure 2C) consisting of the microelectrode array as working electrode, Ag/AgCl ink (C61003P7) as pseudo-reference electrode and carbon ink (C2030519P4) as counter electrode.

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2.5. Electrochemical procedures

2.5.1. Optimisation of the microelectrode array design

After fabrication, the microelectrodes (without CoPC) were examined using cyclic voltammetry to establish the array geometry that produced the optimal microelectrode behaviour. Cyclic voltammetry was performed as outlined in Figure 2B by immersing the three electrode setup into 10 mL of 0.5 mmol L^{-1} ferricyanide solution in PB and scanning the potential from +0.8 V to -0.4 V at a scan rate of 10 mV s^{-1}. A scan rate study was performed between 10 and 100 mV s^{-1} in order to deduce the nature of the microelectrode array behaviour. The response was compared with a standard macroelectrode of area 9 mm^2 (BE2031028D1 supplied by Gwent Group Ltd.).

2.5.2. Evaluation of the mediated microelectrode array

The performance of the base transducer was evaluated by cyclic voltammetry and chronoamperometry in the presence of hydrogen peroxide. Cyclic voltammetry was performed as outlined in Figure 2B by immersing the three electrode setup into 10 mL of 5 mmol L^{-1} hydrogen peroxide solution in PB and scanning the potential from +0.8 V to -0.1 V at 10 mV s^{-1} scan rate. Chronoamperometry was performed as described above for concentrations of hydrogen peroxide in the range 0 to 4 mmol L^{-1}. The potential was stepped from open circuit to +0.5 V, as this potential occurred on the plateau of the voltammogram [24, 25].

2.5.3. Evaluation of the microbiosensor array

In order to evaluate the microbiosensors, chronoamperometry was used to examine concentrations of glucose over the range 2.5 to 20 mmol L^{-1} (45 to 360 mg dL^{-1}). The microbiosensors were examined in the same way as described for the base microelectrodes (Figure 2B). The microbiosensors were immersed in 10 mL of the glucose solution without stirring. After 10 s incubation time, the potential was stepped from open circuit to +0.5 V and the measurement taken after 40 s. Finally, screen printed microbiosensors (Figure 2C) were examined by chronoamperometry by depositing 100 µL of glucose solution and following the above procedure.

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Preferred position for Figure 2

Results and Discussion

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

Initially we examined a range of commercially available dielectric materials in order to insulate the surface of the biorecognition layer. From the study, we identified a suitable formulation (D2060131D1) which produced a uniform and cohesive layer without any pinholes (Figure S1 SI). 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 produced using ultrafast pulsed laser ablation. The size of the holes and the distances between them to produce a microelectrode were chosen based on those found in previous literature [26-28] with at least 10 hemispheres between holes. White light surface profiling was used to visually assess the hole profile and size of the holes (an example is shown in Figure S2 SI). Visual inspection of the holes produced was also carried out under the microscope, as can be seen on Figure 3A and 3B. These images demonstrate that well defined holes could be produced under the fabrication conditions used.

Scanning electron microscopy was also used to assess the size of the hole and the surface of the exposed carbon visually. Figure 3C and 3D shows the SEM images obtained for 40 µm holes by femtosecond laser. The laser beam removed the dielectric layer cleanly to expose the carbon underneath; the carbon/graphite can clearly be distinguished from the dielectric layer. Crystals of salts from the solutions are also visible. It is also important to note that the top of the hole is wider than the bottom. Since we are interested in area of exposed carbon this must be taken into account, especially when looking at the white light images where the carbon layer cannot be distinguished from the dielectric layer from the profiles. This is also important when considering the size of the hole, by using the scale bar on the SEM images, the area of carbon exposed can be calculated more accurately. Since femtosecond pulses produced by laser are much shorter than the time required to heat the substrate (a few picoseconds), the material being processed is removed by rapid vaporisation, avoiding melting and so leaving the surrounding material unaffected for appropriate electrochemical performance.

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The three electrode system used allows for more accurate assessment of the microelectrodes than a two electrode system, this is particularly important since low current signals are expected due to the small areas of the electrodes. The mass transport of redox species to microelectrodes is by radial diffusion; such behaviour is identified by the sigmoidal nature of the resulting voltammograms. However, mass transport of redox species to macroelectrodes is by planar diffusion and is characterised by peak shaped cyclic voltammograms. Table 1 shows a compilation of the microelectrode array configurations and electrochemical performance for the electrodes manufactured on valox using the carbon ink C10903D14 and dielectric ink D2060131D1 and processed using a femtosecond laser (except geometries 17-19 which were to be processed using a picosecond laser). The distances between holes were calculated from the diameter of the hole and the number hemispheres apart. The arrays were chosen by considering the size of the holes, the distances apart and their ability to fit within a 3 x 3 mm area (a standard size used for the macroelectrode). The area of carbon exposed was calculated by multiplying the area of each hole in the array. Figure 4 includes a selection of the configurations examined and the results summarised in Table 1. Figure 4B shows a peak-shaped voltammogram similar to the macroelectrode (Figure 4A), this relates to overlapping diffusion layers on microelectrodes in an array that are spaced too close together displaying overall planar diffusion. On the other hand, Figures 4C and 4D appear to show a transition to sigmoidal behaviour arising from the larger distance between holes.

Microelectrodes produced using the femtosecond laser gave higher current densities than obtained with the picosecond laser with similar electrode geometries (Table 1 and Figure 5). This might be due to the deformation on the edge of the holes observed with the picosecond laser. Consequently, the development of the microbiosensors was carried out using the femtosecond laser ablation.

From Table 1 and Figures 4 and 5 we deduced that the optimum array design comprises 20 μm diameter holes at 180 μm apart produced by femtosecond laser. Under these conditions a well-defined sigmoidal voltammogram was obtained with the highest current density (137.6 μA cm^{-2}). Moreover, steady-state currents were obtained when cyclic voltammetry was performed at different scan rates ranging

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from 10 to 100 mV s^{-1} (Figure 6). Consequently this geometry was investigated for the fabrication of microbiosensors for glucose determination, described in the second part of this paper.

2.7. Design of glucose microbiosensor

Figure 2A shows the chemical reaction that occurs on the surface of the electrode; glucose is converted into gluconolactone and hydrogen peroxide is produced in the presence of the enzyme glucose oxidase. The hydrogen peroxide produced is electrocatalytically oxidised by CoPC, incorporated into the carbon ink, to generate a response current using an applied potential of +0.5 V *vs.* Ag/AgCl (Figure 2A). The next step in the development of the microbiosensor arrays involved an investigation into the electrochemical behaviour of hydrogen peroxide using the microelectrode array (20 µm holes, 180 µm separation).

Cyclic voltammetry was performed to assess the microelectrodes using 5 mmol L^{-1} hydrogen peroxide. In Figure 7A the electrocatalytic oxidation of hydrogen peroxide is observed at a potential of +0.5 V, whereas this response is absent with buffer solution (Figure 7B).

Chronoamperometry was performed with the system shown in Figure 2B. Initially, hydrogen peroxide concentrations up to 4 mmol L^{-1} in the absence of enzyme (*i.e.* with CoPC-SPCE) were investigated in order to demonstrate the effect of increasing concentrations on the electrocatalytic current. Figure 8A shows the chronoamperograms obtained, clearly a linear response was observed over the range studied (slope 0.1431, intercept 0.0293, R^2 0.9933). This behaviour is a pre-requisite for the operation of the glucose microbiosensor.

Secondly, the microbiosensor (*i.e.* CoPC-SPCE with immobilised GOX) array was evaluated in the presence of glucose. Figure 8B shows the resulting chronoamperograms obtained; the inset shows that an increase in response was obtained up to 20 mmol L^{-1} (360 mg dL^{-1}) of glucose, the relative standard deviation (RSD) ranged from 15 to 20% for the different concentrations tested (slope 0.1175, intercept 0.5289, R^2 0.9363,). The limit of detection (3.3 mmol L^{-1}, 59.4 mg dL^{-1}) was estimated as the concentration corresponding to three times the standard deviation

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of the blank added to the blank signal. In both cases, hydrogen peroxide and glucose determination, steady state currents were observed.

After demonstrating the potential of the microbiosensor array to measure analytically relevant glucose concentrations, the final step pursued in this work was to evaluate the microbiosensor array integrated into a screen printed three-electrode configuration (Figure 2C). As shown in Figure 8C, a linear response was obtained over the glucose concentration range studied for the glucose microbiosensor (slope 0.1547, intercept 0.5139, R^2 0.9872); this was compared with the conventional glucose macrobiosensor (slope 0.2053, intercept 0.6007, R^2 0.9925). Clearly, the response obtained is linear for both over the range studied with only 0.05 μA/mmol L^{-1} difference in sensitivity. RSD was found to be ranging from 0.9 to 6.9% (microbiosensor) and from 0.7 to 12.9% (macrobiosensor) for the different concentrations tested. The limit of detection (0.7 mmol L^{-1}, 12.6 mg dL^{-1} for the microbiosensor, and 1.1 mmol L^{-1}, 19.8 mg dL^{-1} for the macrobiosensor) was estimated as the concentration corresponding to three times the standard deviation of the blank added to the blank signal. The limit of quantification estimated as the concentration corresponding to ten times the standard deviation of the blank added to the blank signal was found to be 5 mmol L^{-1} (90 mg dL^{-1}) in both cases, micro and macrobiosensor. Consequently, these promising results show that either of these biosensors with a dynamic range up to 20 mmol L^{-1} (360 mg dL^{-1}) might be suitable for clinical monitoring (normal range before a meal is 3.6 to 5.5 mmol L^{-1}, 65 to 100 mg dL^{-1}, after meals up to 8 mmol L^{-1}, 145 mg dL^{-1}, and in any cases less than 10 mmol L^{-1}, 180 mg dL^{-1}). However, one of our future goals is to apply these microbiosensors for continuous monitoring cell culture growth and metabolism in bioreactor or cell culture systems, in unstirred solutions (*e.g.* monitoring of sugars, amino acids, vitamins, enzymes, nutrients or metabolites); for these purposes, where steady-state currents are required, only the microbiosensor array would be suitable.

3. Conclusions

This paper has demonstrated, for the first time, a new method for the fabrication of microelectrode arrays and microbiosensor arrays based on the combination of screen-printing and laser ablation technologies; in the latter case, an enzyme

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containing carbon ink, with a dielectric coating, underwent laser ablation, which did not affect the enzyme activity. Importantly, this procedure has potential for the simple fabrication of many types of microbiosensor by incorporating an appropriate enzyme into the ink carbon formulation. As far as we are aware, this is the first time that ultrafast pulsed laser ablation has been used to drill holes through a dielectric, into a carbon ink containing an enzyme. This technology allows rapid and easy modification of the hole size and array geometry with good reproducibility. We believe that this generic platform has wide applications in biomedical, pharmaceutical, agri-food and environmental sectors.

Appendix A. Supplementary Information

Figures S1 and S2

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BIOGRAPHIES

We will submit the rest of the author biographies offline.

Susana Liébana Girona, graduated in Chemistry at Autonomous University of Barcelona (UAB), Catalonia (Spain) (2007) and PhD in Chemistry from the same university (2013). During her pre-doctoral time in Sensors and Biosensors Group (UAB) she carried out research on the development of electrochemical transducers and bioanalytical strategies based on nanobiomaterials and magnetic particles for food safety applications. Currently, she is a postdoctoral researcher for Applied Enzyme Technology Ltd. based at The Gwent Group (UK) since 2013. Her research is focused mainly on the integration of new materials into biosensors for diagnostic tests within the European Marie Curie ITN PROSENSE.

John P. Hart received his MSc in Analytical Chemistry and PhD in Electroanalytical Chemistry from Chelsea College, University of London, UK. He is Professor in Biosensor and Electroanalytical Sciences in the Faculty of Health and Applied Sciences, University of the West of England, Bristol, UK. His current research interests include the design, development and application of disposable amperometric sensors and biosensors, based on screen-printing technology, for environmental, agri-food, and biomedical applications and gas detection.

Guido A. Drago, graduated from University College London with a PhD in Genetics in 1992. He carried out his postdoctoral training in Cardiac Biochemistry at the University of Leeds and the University of Maryland (USA). He joined Applied Enzyme Technology Ltd. in 1999 as a research officer on a European project called PROMOFILM. He became Managing Director of AET in 2001. He became Director

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of Gwent Electronic Materials Ltd. part of the Gwent Group in 2012. His technical expertise lies in the stabilisation, immobilisation and purification of enzymes and antibodies used in the construction of novel biosensors.

Walter Perrie is Senior Research Fellow in the School of Engineering, University of Liverpool. His research interests include ultrafast laser micro-machining of solid surfaces and structuring of photonic components inside transparent polymers, areas in which he has published extensively.

FIGURE CAPTIONS

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

Figure 2. Reaction pathway involved in the operation of the glucose microbiosensor (A). Diagram of electrochemical setup based on three electrodes using external reference and counter electrode (B) and screen printed electrodes (C).

Figure 3. Microscope images at 60X (A) and 200X (B) magnification of the microelectrode array produced by femtosecond laser with 20 µm holes at 180 µm distance apart. Scanning Electron Microscopy images of the 40 µm holes produced by femtosecond laser (C and D).

Figure 4. Cyclic voltammograms using as working electrodes the macroelectrode (A) and microelectrode arrays produced by femtosecond laser corresponding to: Geometry 8, 40 µm holes, 60 µm distance apart (B); Geometry 9, 40 µm holes, 110 µm distance apart (C), and Geometry 10, 40 µm holes, 160 µm distance apart (D) (n=3).

Figure 5. Cyclic voltammograms using as working electrodes the microelectrode array with 40 µm holes at 160 µm distance apart produced by femtosecond laser (Geometry 10, red) and picosecond laser (Geometry 17, black) (A). Microscope images at 60X magnification for comparison between the processing of microholes (20 µm) using the femto (B) and picosecond (C) laser.

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Figure 6. Cyclic voltammograms using as working electrodes the macroelectrode (A) and the microelectrode array with 20 µm holes at 180 µm distance apart produced by femtosecond laser (B).

Figure 7. Cyclic voltammograms using as working electrode the microelectrode array with 20 µm holes at 180 µm apart. A volume of 10 mL of PB (green) and 5 mmol L^{-1} hydrogen peroxide solution in PB (blue) was used.

Figure 8. Chronoamperograms and calibration plot for microelectrodes obtained with 10 mL of (A) hydrogen peroxide solutions and (B) glucose solutions using external reference and counter electrodes, and (C) calibration plot obtained for 100 µL of glucose solutions using the macroelectrode (blue) and microelectrode (red) screen printed three-electrode configuration (n=3).

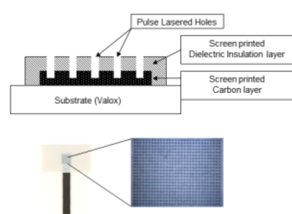

Fig. 1

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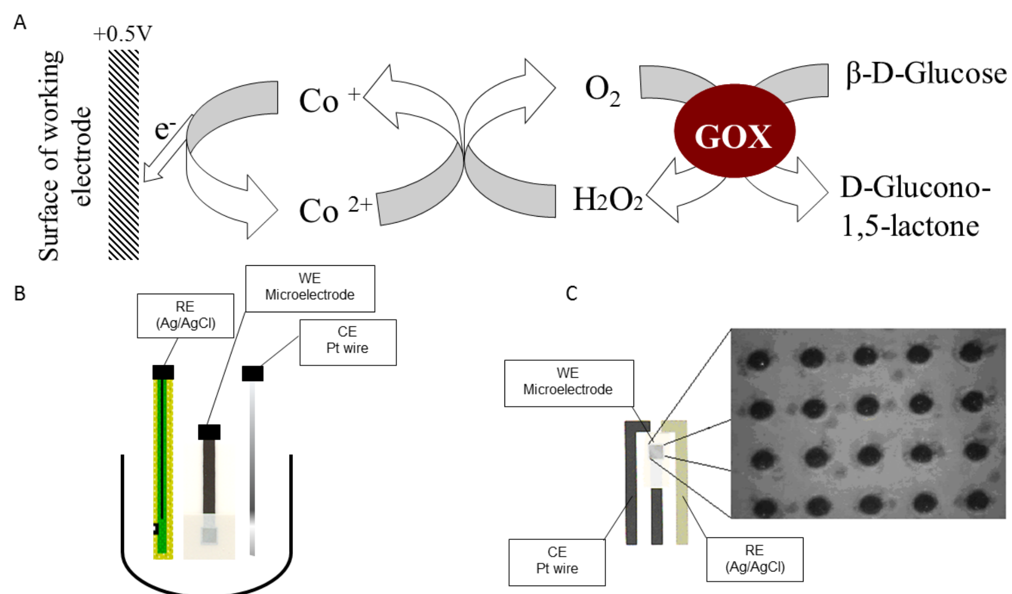

Fig. 2

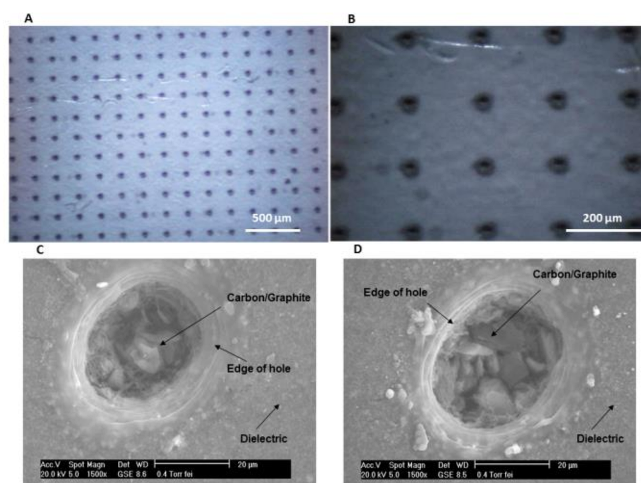

Fig. 3

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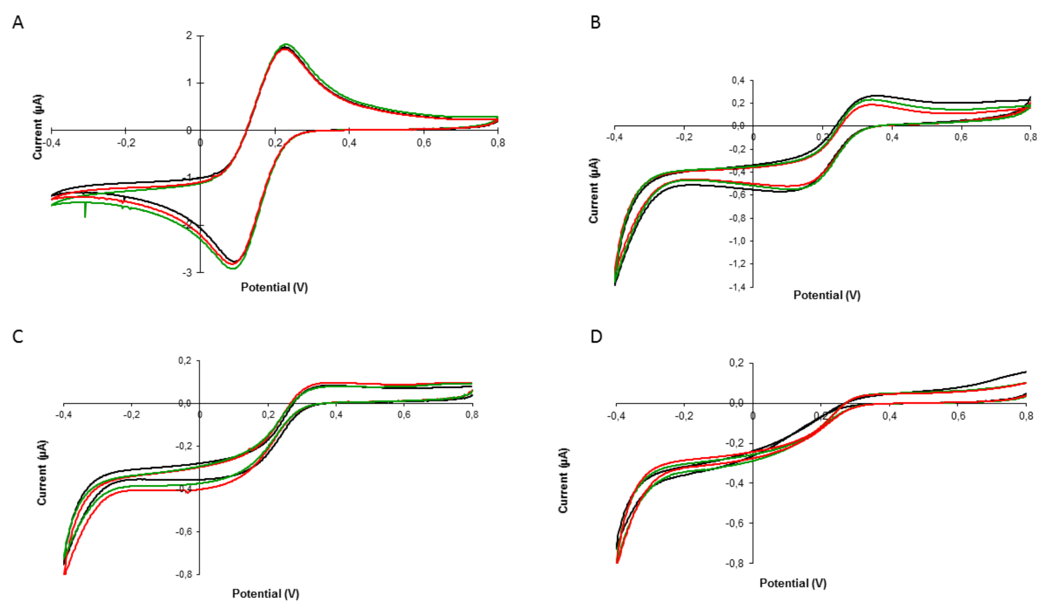

Fig. 4

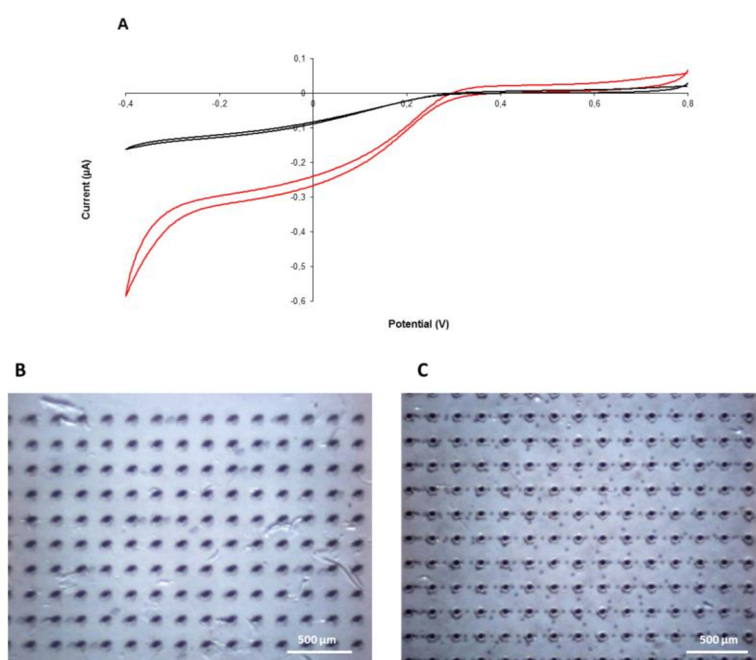

Fig. 5

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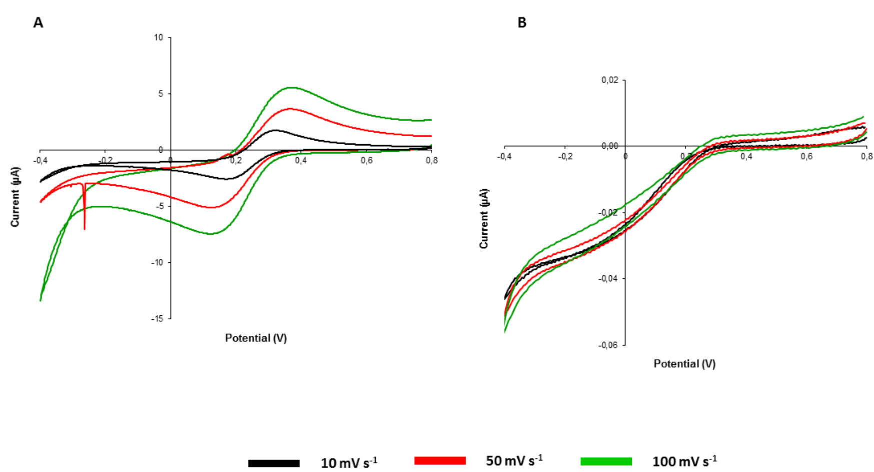

Fig. 6

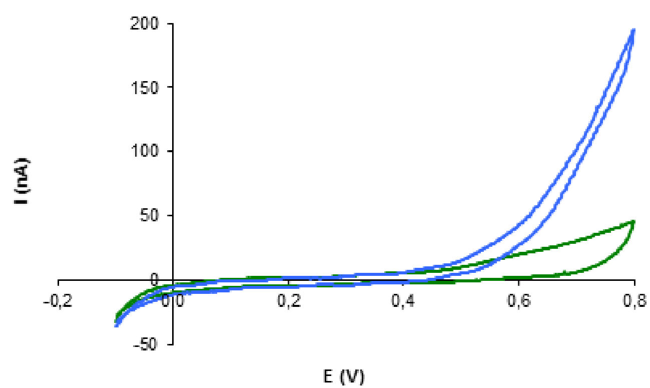

Fig. 7

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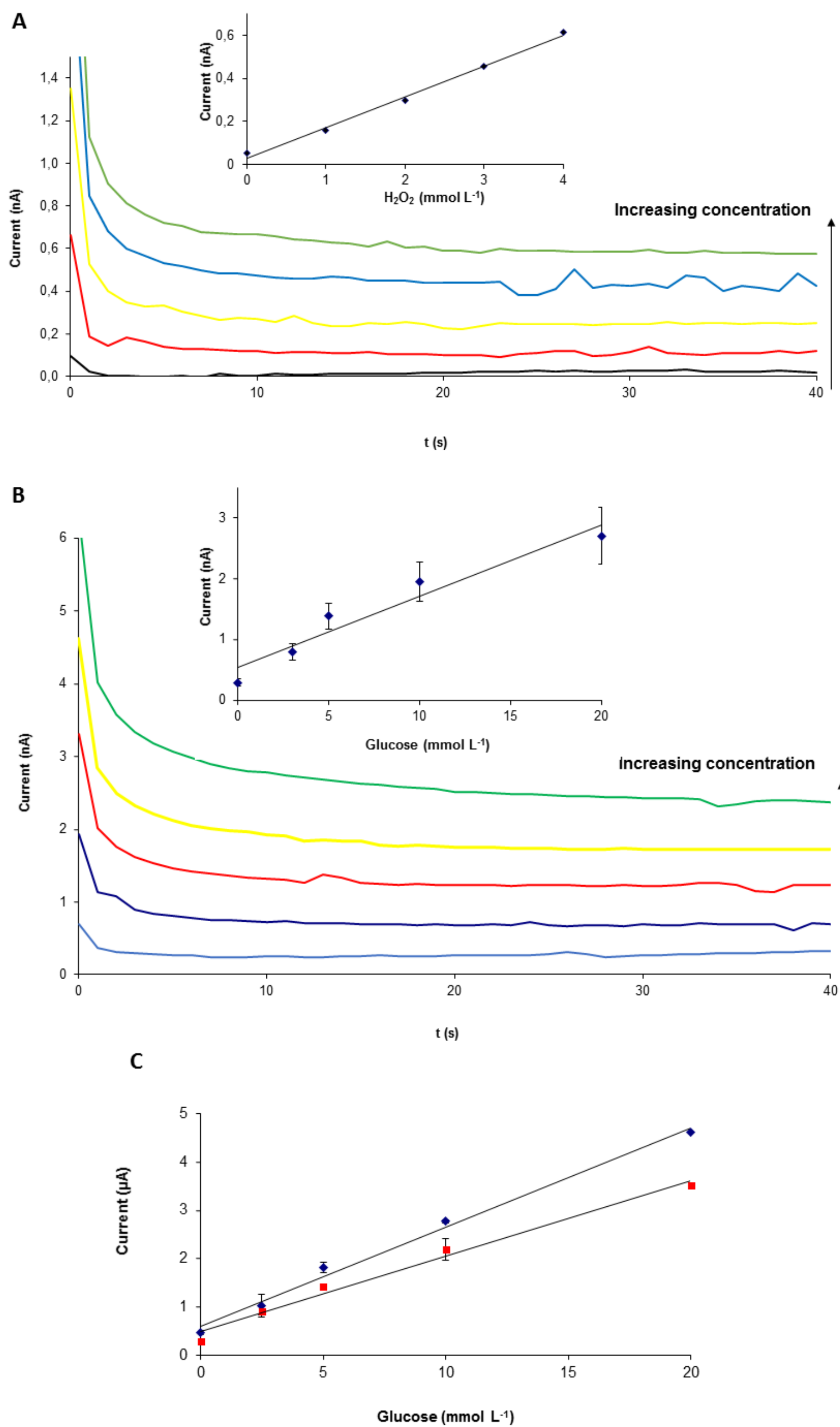

Fig. 8

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Table 1. Compilation of microelectrode arrays configurations and electrochemical performance.

Geometry	Laser	Diameter of holes (µm)	Distance apart (µm)	Array	Total number of holes	Area of carbon exposed (cm^2)	Current density (µA cm^{-2})	Voltammetric shape
1	femto	0	0	Macroelectrode (3 x 3 mm)	0	9.00 e-2	31.43	peak-shaped
2	femto	20	30	60 x 60	3600	1.13 e-2	55.11	peak-shaped
3	femto	20	55	40 x 40	1600	5.02 e-3	93.77	sigmoidal
4	femto	20	80	30 x 30	900	2.83 e-3	96.43	sigmoidal
5	femto	20	180	15 x 15	225	7.07 e-4	137.59	sigmoidal
6	femto	20	280	10 x 10	100	3.14 e-4	129.65	sigmoidal
7	femto	20	480	6 x 6	36	1.13 e-4	99.79	sigmoidal
8	femto	40	60	30 x 30	900	1.13 e-2	53.00	peak-shaped
9	femto	40	110	20 x 20	400	5.04 e-3	82.98	peak-shaped
10	femto	40	160	15 x 15	225	2.84 e-3	91.57	sigmoidal
11	femto	40	560	5 x 5	25	3.15 e-4	102.29	sigmoidal
12	femto	40	160	15 x 15	225	2.84 e-3	74.81	sigmoidal
13	femto	40	160	15 x 15	225	2.84 e-3	63.92	sigmoidal
14	femto	20	180	19 x 19	361	1.13 e-3	73.80	sigmoidal
15	femto	20	280	13 x 13	169	5.31 e-4	96.30	sigmoidal
16	femto	60	540	5 x 5	25	7.07 e-4	59.70	sigmoidal
17	pico	40	160	15 x 15	225	2.84e-3	52.57	sigmoidal
18	pico	30	160	15 x 15	225	1.59 e-3	59.79	sigmoidal
19	pico	20	160	15 x 15	225	7.07 e-4	42.15	sigmoidal