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# An HRP-based amperometric biosensor fabricated by thermal inkjet printing

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**Abstract**

Direct inkjet printing of a complete and working amperometric biosensor for the detection of hydrogen peroxide, based on horseradish peroxidase (HRP), has been demonstrated. The device has been realized with a commercial printer. A thin layer of PEDOT:PSS, which was in turn covered with HRP, was inkjet printed on top of an ITO-coated glass slide. The active components of the device retained their properties after the thermal inkjet printing. The whole device has been encapsulated by means of a selectively permeable cellulose acetate membrane.

The successful electron transfer between the PEDOT:PSS covered electrode and the enzyme has been demonstrated, and the biosensor evidenced very good sensitivity, in line with the best devices realized with other techniques, and a remarkable operational stability. This result paves the way for an extensive application of "biopolytronics", i.e. the utilization of conductive/semiconductive polymers and biologically active molecules to design bioelectronic devices using a common PC, and exploiting normal commercial printers to print them out.


*Keywords:* Thermal inkjet printing; Biological ink; Electronic ink; Bioelectronics; Biopolytronics; Biosensors

## 1. Introduction

Inkjet printing has many new practical applications, such as for example the production of printed electronic circuits. This latter application is of particular interest, due to the evident practical advantages of this approach. It permits infact to achieve shorter process times, higher rates of active material utilization, and a great versatility [1,2]. Among the various technologies, thermal, piezoelectric and electrostatic printing are the most diffused ones [3,4]. In the first technology, heat-generated vapour bubbles are exploited to eject ink droplets out of a chamber, in the second one the driving force for the ink ejection is provided by a piezo-electric actuator, while in the third one the ink is ejected following the application of a strong local electric field.

Another advantage of inkjet printing is the easiness of management of the digital image; in addition, the absence of contact between the printhead and the substrate makes this technique particularly useful for contact-sensitive surfaces. Today, the inkjet printing has already been used to fabricate all-polymer transistors [5,6], OLEDs [7], biosensors [8,9], arrays of bacteria colonies [10], biochips [11], to perform DNA synthesis [12], for the microdeposition of active proteins [13], and for free-form fabrication techniques aimed at the creation of acellular polymeric scaffolds [14].

On the basis of the cited technical possibilities, our research group has shown recently that it is possible to realize printed bioelectronic devices by means of thermal inkjet technology [9], printing enzymes such as GOD and β-galactosidase [15] and conjugated polymers like poly(3,4-ethylenedioxythiophene/polystyrene sulfonic acid) (PEDOT/PSS) [16] without appreciable degradations of the specific functions of the organic molecule. This approach to the realization of bioelectronic devices has been named "biopolytronics", linking polymer electronics and biological molecules by means of digitally controlled direct printing of dot matrices on a substrate, using inkjet technology with electronic and biological inks. These devices are in fact characterized by the electronic transport through the different active printed

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layers/dots; in particular, the electronic transport from an active enzyme (Glucose Oxidase) to a conductive polymer (PEDOT:PSS) has been demonstrated [9].

In order to investigate the opposite electronic transport direction possibility, i.e. from the electronically conductive polymer to the enzyme, we present in this work a biosensor fabricated through the sequential deposition of an electronic ink containing the conductive polymer blend PEDOT/PSS and a biological ink containing the enzyme HRP, both deposited by thermal inkjet technology, onto an ITO-coated glass. HRP is infact characterized by using electrons for catalyzing the reduction of hydrogen peroxide, a step which requires an efficient electron transfer from the electrode to allow the enzyme to fully develop its activity. In order to preserve PEDOT/PSS and peroxidase from dissolving in water, the printed biosensor was protected with a cellulose acetate membrane, applied by dip-coating.

## 2. Experimental

### 2.1. Chemicals and materials

Poly(3,4-ethylenedioxythiophene/polystyrene sulfonic acid) 1.3 wt.% dispersion in water, polyoxyethylene-(20)-sorbitan monooleate (Tween 80), ethylenediaminetetraacetic acid, tetrasodium salt hydrate (EDTA) 98%, tetrahydrofuran 99%, potassium nitrate 99%, ferrocenemethanol 97%, peroxidase (HRP: EC 1.11.1.7. from horseradish, 133 U/mg), acetone (99.5%), 3-metil,2-benzothiazolinone hydrazone (MBTH) were purchased from Sigma–Aldrich. Cellulose acetate ($M_n$ 29,000) and guaiacol were purchased from Fluka and Merck, respectively. Phosphate buffer 0.1 M (pH 6.5) and 0.02 M (pH 7.5) was prepared according to normal laboratory procedures. Brilliant blue FCF (E133) were purchased from Fiorio (Italy) and ITO-coated glass plates (12 Ω/m$^2$) were purchased from Technopartner (Italy).

### 2.2. Instruments

Spectrophotometric measurements were performed with a UV–vis scanning spectrophotometer (Uvikon 923, Bio-teck Instruments srl, Milano). The electrochemical measurements were performed with an Autolab PGSTAT20 (Ecochemie, Utrecht, The Netherlands) potentiostat/galvanostat interfaced with a personal computer. The thickness of the films was evaluated by atomic force microscopy (AFM, Scanning Probe Microscope Vista 100, Burleigh Instruments Inc.), using a long-range 100 Å tip, operating in contact mode with a 10 nN force constant.

### 2.3. Methods

#### 2.3.1. Biological ink preparation

The biological ink was obtained dissolving 1.7 mg/mL of HRP in a 0.1 M phosphate buffer, pH 6.5, which contained EDTA 1.5 mM as antimicrobial agent and 10% (w/v) of glycerol as stabilizer.

#### 2.3.2. Electronic ink preparation

The electronic ink was prepared diluting 20 mL of the 1.3 wt.% PEDOT/PSS dispersion in distilled water, to a final volume of 50 mL, and filtering the obtained dispersion with a 0.25 μm filter (cellulose acetate). In order to obtain the necessary surface tension for the printing device, the dispersion was added of 0.426 g of Tween 80 (6.50 mM solution).

#### 2.3.3. Description of the printing system

A commercial inkjet printer Canon i905D with a thermal printhead was used for printing the electronic and biological inks. The configuration of the system permitted to realize matrices on solid supports, with a resolution up to 4800 × 1200 d.p.i., in which each dot was formed by an ejected ink drop of about 2 pL. The cartridge feeding the printhead was filled with the biological or the electronic ink, and layers/patterns printing was performed using a commercial software (CD label print). The ink deposition was realized setting the printing quality to "standard".

#### 2.3.4. Enzyme activity assay

The HRP activity was tested with an assay based on previous work [17] relying on oxidative coupling between MBTH and guaiacol. To 3 mL of phosphate buffer 0.02 M, under stirring, 0.1 mL of HRP 1.7 mg/mL (192 U/mL) and 0.5 mL of guaiacol 0.1 M were added, followed by 0.5 mL of MBTH solution (7.4 mM) and 0.02 mL of hydrogen peroxide (500 mM). After the addition, the reaction was allowed to proceed for three minutes, under stirring, at 25 °C, then stopped adding to the mixture 0.5 mL of a 2N solution of $H_2SO_4$ and 1 mL of acetone. The so-formed red complex was analyzed by UV–vis spectrophotometry at 505 nm.

#### 2.3.5. Determination of the deposited enzyme

The amount of printed enzyme was determined realizing preliminary tests adding to the biological ink (containing 1.7 mg of HRP) 1.2 mg/mL of brilliant blue FCF (E133). The printing support was a hydrophobic polyester sheet on which the biological ink was not adsorbed. The printer was then connected to a standard PC and a filled rectangle (with an area of 8 cm$^2$), defined by a word-processing software at a standard resolution, was printed on the substrate. The deposited ink was recovered washing off with 10 mL of water and the amount of printed E133 was determined spectrophotometrically at 628 nm, having in this way and indirect assessment of the amount of printed enzyme.

#### 2.3.6. Electrochemical measurements

A three electrode cell geometry was used in chronoamperometric experiments. The counter electrode was a Pt wire, the reference electrode was a SCE, while an ITO-glass inkjet printed device was used as the working electrode. The response of the inkjet printed electrode was measured by chronoamperometry, dipping the electrode in 25 mL of a stirred buffer solution (0.1 M phosphate buffer + 0.1 M $KNO_3$, pH 6.5) in presence of 14 mg/L ferrocenemethanol (FcMeOH), as mediator, at an applied potential of −0.10 V. After a stable current background was reached (30–60 s), aliquots of 250 μL of a 25 mM hydrogen peroxide