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# Thermal Inkjet Technology for the Microdeposition of Biological Molecules as a Viable Route for the Realization of Biosensors

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## ABSTRACT

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Recent progress in inkjet printing of parts of biosensors are highlighted, with particular reference to the printing of biologically active molecules. We describe a system constituted by a thermal inkjet printer, adapted to layering a bidimensional array of dots [701 × 701 dots per inch] on

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solid supports. The printer was used to depose a $\beta$-galactosidase (GAL)-containing ink on a polyester sheet, with dots obtained from 10 pL drops, each drop containing in turn 6 pg of enzyme. The activity of GAL after the preparation was determined using a colorimetric probe (Brilliant Blue FCF). The activity loss of the microdeposed enzymes was found to be around 15%, showing that the 2 μsec-lasting thermal shock experienced by the biomolecule into the printhead nozzle affects to a lesser extent the activity of the thermal inkjet deposited enzyme. In conclusion, the most recent findings of our group in this line are depicted, and a view of possible future developments of the “biopolytronics” field is outlined.

*Key Words:* Thermal inkjet printing; Biological ink; $\beta$-Galactosidase; Brilliant Blue FCF; Biopolytronics; Biosensor.

## INTRODUCTION

In the last years the field of biosensors has grown in terms of technological and theoretical achievements for the realization of effective devices for clinical, environmental, and industrial analysis.[1]

Basically, a biosensor is a device having a biological sensing element either intimately connected to, or integrated within, a transducer. In the most recent devices of third generation, the sensing element is directly bound to the electronic system that transduces and amplifies the signal.[2]

The biological element, which is typically a redox enzyme, and the electronic transducers are, however, foreign components with respect to each other, leading to the lack of electrical communication among them, as highlighted by the Marcus equation.[3] The integration of the biological sensing elements within electronically active materials represents the most promising solution to this problem, and it is generally achieved using physically blended composites, such as redox-enzymes/carbon paste blends. Another bottleneck in biosensor technology lies in the increasing miniaturization of the electronic supports, reaching at the limit the nanoscale, and in the necessity of multifunctional devices, that in turn requires the development of new microdeposition techniques possessing high efficiency and precision.

On these premises one of the most used techniques for the production of enzyme-based sensors is screen printing, where a liquid medium is forced through a mesh screen to form an image onto a surface.[4] Parts of the screen are blocked off (“masked”), thus preventing the medium from passing through. The “mask” is a negative of a part or the whole of the desired image, and complex images may be built up through the use of successive

screens. This technique has been used to produce thick films constituting the different parts of a biosensor, by mean of electronical and biological paste inks, and allows to obtain resolutions as high as 390 dots per inch (dpi), with a dot diameter around 40–70 μm. Miniaturization, versatility, low cost, and the possibility of mass production makes the screen printing a good technique for a large scale biosensors production. The screen-printing technology requires, however, the previous preparation of masks, that have to be designed for any single biosensor, and have to be kept at direct contact with the device support, with the consequent risks of damaging the whole device. As other traditional printing processes such as lithography and flexography, screen printing belongs to the class of impact printing processes. In contrast, it is possible to apply the ink to a substrate without a physical impact, or pressure, between the printing device and the support, via a variety of devices termed under the collective name of non-impact printers.[5]

An appealing alternative to the screen-printing technique is hence the inkjet printing, in which the ink is sprayed from an array device (printhead) employing a sequence of nozzles. The most common type of this device is represented by the drop-on-demand printheads using thermal or piezoelectric technology.[6]

In the latter one, crystalline materials undergoing mechanical stress under an applied electrical field are exploited. A minute contraction or expansion of these materials, confined into the nozzle, allows for the reduction of the space available for the ink, thus increasing the pressure and causing the ejection of a drop through the orifice.

In the thermal technology, on the other hand, the ink ejection is obtained thanks to a vapor bubble that is formed on the surface of a heating resistor, which is located inside each nozzle constituting the array device. During the heating, the vapour bubble grows, increasing the pressure inside the nozzle, and thus forcing a droplet of ink through the orifice. As the heat is suddenly cut off, the bubble breaks and the pressure into the nozzle decreases, thus drawing in the ink from the reservoir.

For the fabrication of biological molecule microarrays, mechanical and piezoelectric microspotting are usually employed, due to the lack of heat sources in these devices. In fact, one of the expected major problems in using thermal inkjet technology is the exposure of the biological elements to high temperatures (200–300°C) and to the associated shearing stress ($10\,\mathrm{msec}^{-1}$), which may be able to degrade the biomolecules.

In general, the inkjet technology allows to obtain resolutions even higher than 1200 dpi and a mean dot diameter of about 15–40 μm, moreover, as mentioned above, the absence of direct contact between the printing head and the biosensor support, due to the lack of an expansive cliché, is a further advantage with respect to other deposition techniques.

Despite these advantages, at the moment few works exploiting this technology for the preparation of parts of, or of potential constitutents of biosensors are described in the literature. For example, Turner and co-workers reported an inkjet printing system in which different enzymes were applied, while the pattern of the electrode base was designed by the screen-printing technique (Fig. 1).[7] Electrostatic inkjet printing (i.e., a stream of droplets, passing between two charged plates, is directed into a pattern by varying the electrical potential applied to the plates) was used in this work. In 1996 Hart et al.[8] demonstrated an amperometric enzyme electrode for lactate, in which the outer and the inner membranes were applied by piezoelectric inkjet printing. Okamoto et al.[9] have instead developed a microarray fabrication method using a thermal inkjet technology to eject 5′-terminal-thiolated oligonucleotides onto a glass surface, while in 2000 Roda et al.[10] have

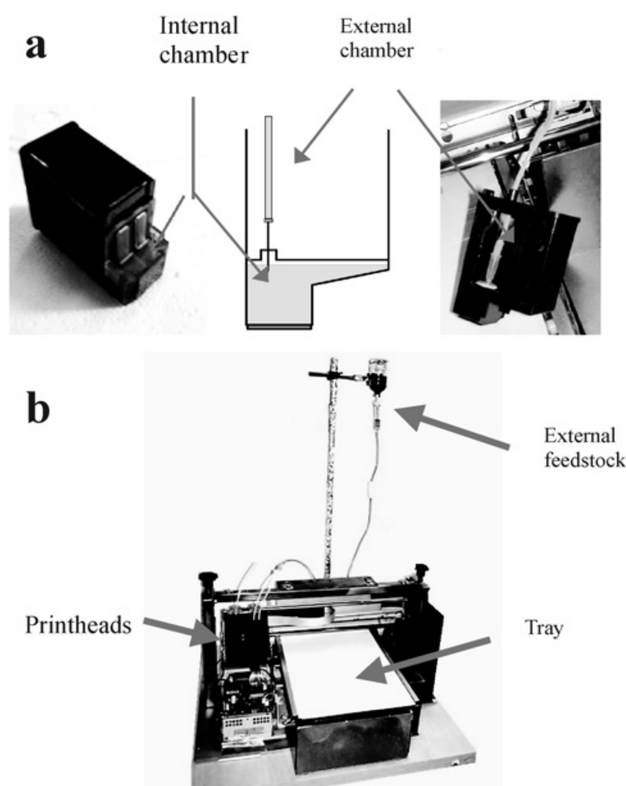


*Figure 1.* (a) Modified Olivetti I-Jet (Italy) printhead; (b) Tiljet printer (Lesepidado, Italy) for printing on solid plates. (*View this art in color at www.dekker.com.*)

demonstrated the possibility to make a protein microdeposition of horseradish peroxidase on cellulose using a conventional thermal inkjet printer, without however assessing if any degradation occurred in the enzyme consequent to the deposition.

In this view, we are currently exploring the possibility of employing the thermal inkjet microdeposition in order to develop cheap, reliable, and printable-on-demand microsized hybrid biosensors. In fact, though the thermal deposition step is expected to affect negatively the activity of biological molecules, to our knowledge no clear assessment of such a deactivation has ever been made. In this paper, we have then described a new rapid method for investigating the efficiency of the enzyme microdeposition using an inkjet technology. $\beta$-Galactosidase (GAL) from *Aspergillus oryzae* was here used for testing the method and evaluating the effect of thermal inkjet deposition on the printed enzyme's activity.

## EXPERIMENTAL PART

### Preparation of the Biological Ink

GAL from *A. oryzae* (Sigma, EC.3.2.1.23, 11.7 U/mg) was purchased from Sigma-Aldrich (Germany). The biological ink was obtained by dissolving 0.6 mg/mL of GAL in 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.

### Enzyme Activity Assay

The activity of the enzyme was assayed by following the rate of formation of free *o*-nitrophenol from *o*-nitrophenyl-$\beta$-D-galactopyranoside (ONPG).[11]

A 2.2 mL of 0.1 M phosphate buffer, pH 6.5, were mixed with 0.25 mL of ONPG ($5 \times 10^{-2}$ M in distilled water) and 50 μL of enzyme solution, and the mixture was incubated at 30°C. The reaction was stopped at controlled times by adding 0.5 mL of 1.0 M of sodium carbonate. The *o*-nitrophenol released by the action of the enzyme was determined spectrophotometrically at 420 nm.

### Description of the Printing System

The inkjet printer, based on thermal inkjet technology from Olivetti Tecnost (Ivrea, Italy), was developed in a prototype model by Lesepidado srl (Bologna, Italy) for printing on solid surfaces [Fig. 1(b)]. The biological

ink was ejected using a printhead supplied by Olivetti I-Jet (Aosta, Italy) which is characterized by 208 nozzles having a roof-shooter design. This drop-on-demand technology permits production of a matrix on a solid support up to $1200 \times 1200$ dots per inch, in which each dot is formed by an ejected ink drop of 10–12 pL. The cartridge was filled with the biological ink using an external flask connected with the printhead through a feeding tube [Fig. 1(a)].

### Determination of the Sample Deposition

The biological ink was mixed with 1.2 mg/mL of Brilliant Blue FCF, a food dye (E133) purchased by Fiorio (Italy). The pattern of the microdeposed biological ink was a square having a resolution mode at $1200 \times 1200$ dpi with a dimension of 154 $cm^2$, defined by a standard word-processing software. The printing support was a hydrophobic polyester sheet on which the biological ink was not adsorbed. After the printing, the microdeposed ink was recovered by washing the sheet printed surface with 3 mL of 0.1 M phosphate buffer. The recovered E133, and in turn the volume of ejected ink, was determined spectrophotometrically at 628 nm.

## RESULTS AND DISCUSSION

A biological ink was produced starting from GAL in phosphate buffer, using EDTA as antimicrobial agent and glycerol as stabilizer and ink viscosity regulator, in order to achieve the best possible performances from the thermal inkjet printer. A food dye (Brilliant Blue FCF, E133) was added to the mixture as a colorimetric probe, in order to visualize the printed area and to spectrophotometrically determine the volume of the ejected ink. E133 was chosen by reason of its being a highly stable dye both at high temperature and different pH. Moreover, this dye presents a spectral profile having the absorption maximum in the range 500–700 nm (with the peak at 628 nm), with a high absorption coefficient of 112,662 $M^{-1} cm^{-1}$. It is hence very suitable for spectrophotometric purposes, and it does not interfere with *o*-nitrophenol, released from the hydrolysis of ONPG in basic conditions, which has the maximum absorption at 420 nm (Fig. 2). Finally, its effect on the enzyme activity was found to be not significant (data not shown), thus permitting a reliable spectrophotometric determination of the GAL activity.[11] EQ1

The printer used to deposit the enzyme was modified in order to permit easy operations. In particular, the application of an external feedstock (Fig. 1), vertically adjustable thanks to a suitable arm, permitted to control the pressure inside the ink chamber, compensating imperfect reological

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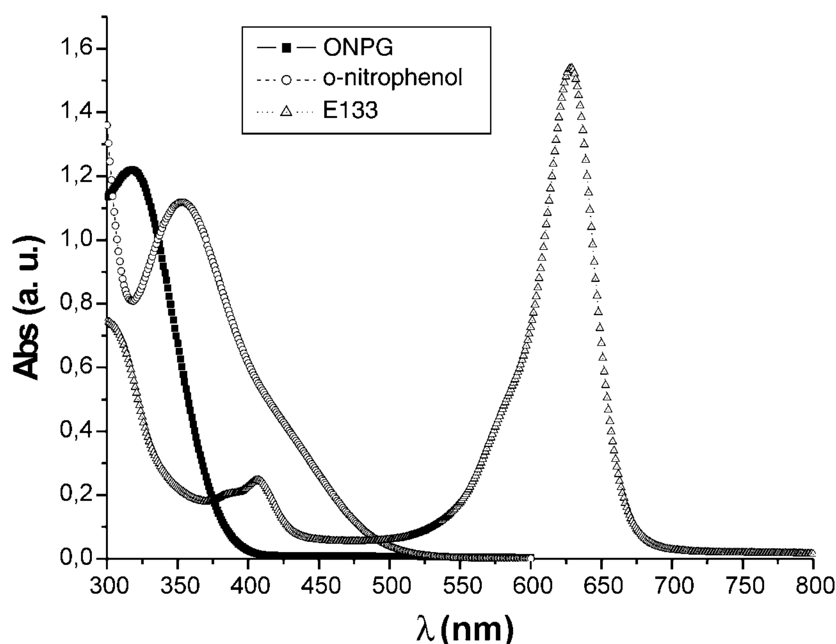


***Figure 2.*** Spectrophotometric profiles of ONPG (■), *o*-nitrophenol (○), and Brilliant Blue FCF (E133) (△) in the basic conditions realized for the GAL activity assay.

parameters of the ink, such as surface tension and viscosity, which are of capital importance in determining the proper operation of the printing device.

After different tests, the best printing support was identified in polyester transparent sheets. They present high hydrophobicity and negligible interaction with the ink components, permitting thus a quantitative recovery of the printed E133 and GAL.

To perform the printing, the polyester sheet was put on the mobile flat tray, which moved perpendicularly with respect to the motion direction of the printing bar. The distance between the nozzle array and the support was 2–4 mm, and the biological ink was put into the external feedstock, which was connected to the printhead by a plastic cannula. The printer was then connected to a standard PC and a filled rectangle (with an area of $154\,cm^2$), designed with a standard word-processor, was printed on the substrate.

The volume of the recovered biological ink was calculated via spectrophotometric determination. Since for the realization of the square pattern of $154\,cm^2$ it was necessary to use $0.76\,\mu L/cm^2$ (or $4.92\,\mu L$ per square inch), and as the volume of each drop ejected (containing 6 pg of enzyme) through

295 a single nozzle was 10 pL, the printhead had ejected 491,861 dots per square
296 inch. This means that the actual printing resolution achieved was
297 701 × 701 dpi, which is a quite low value with respect to the nominal soft-
298 ware-selected one of 1200 × 1200 dpi. This lower resolution was essentially
299 due to the scarce efficiency of the printhead, in which some nozzles were
300 most likely clogged or not available. A better formulation of the ink, together
301 with other side devices (better manipulation of the ink cartridge, etc.), should
302 improve this result, that is however quite interesting when compared to the
303 present resolutions achieved by screen printing.
304 The activity loss of the microdeposed enzyme after the ink ejection was
305 evaluated through a spectrophotometric assay (see Experimental part for
306 details), exploiting the different maximum absorption wavelength of E133
307 (620 nm) and of sodium *o*-nitrophenolate (420 nm). The result showed that
308 only around 15% of the enzyme activity was lost after the printing, i.e., no
309 significant denaturation effects due to the printhead heater were evidenced.
310 We ascribe this finding to the fact that the heater into the nozzle generates a
311 bubble of fluid at high pressure (>10 atm), which in turn presents a gradient
312 of decreasing temperature from the surface of the heater to the bulk of the ink
313 solution (Fig. 3). This gradient permits the enzyme in the bulk to feel an actual
314 temperature not as high as the one on the surface of the resistor, decreasing
315 with the time elapsed from the initial heating event. In fact, the drop-on-
316 demand mechanism comprehends a first phase with the formation and the col-
317 lapse of the bubble in 10 μsec, and then a second one in which the nozzle is
318 refilled in 80–200 μsec. Moreover, the bubble formation is essentially a
319 "shot" having a thermal flash-time around 2 μsec, and this factor may contrib-
320 ute to a negligible degradation. Also the glycerol present in the ink, though
321 intended as a wetting agent for avoiding the clogging effect on the external
322 surface of the nozzle, probably plays a role in increasing the enzyme stability
323 to the thermal shock. In fact, a stabilizing effect of the glycerol at atmospheric
324 pressure against thermal deactivation of the *K. lactis* GAL was already
325 evidenced by Athés et al.[12,13] Such an effect is explainable considering
326 that the interactions occurring between the polyol and the protein, and in par-
327 ticular with the active site, may lead to the displacement of some water mol-
328 ecules surrounding the protein molecule. Finally, it has to be considered also
329 that the manipulation step needed for assessing the residual enzyme activity
330 may have contributed to diminish the latter, so the actual value of degradation
331 could be even lower than the found 15%.
332 These data demonstrate that a commercially available thermal inkjet
333 technology can be adapted to protein microdeposition, and that the use of a
334 colorimetric probe incorporated in the biological ink permits the determination
335 of the yield of the ejected enzyme activity. On this basis the thermal step of
336 the ink ejection does not represent a limitation for the protein microdeposition;

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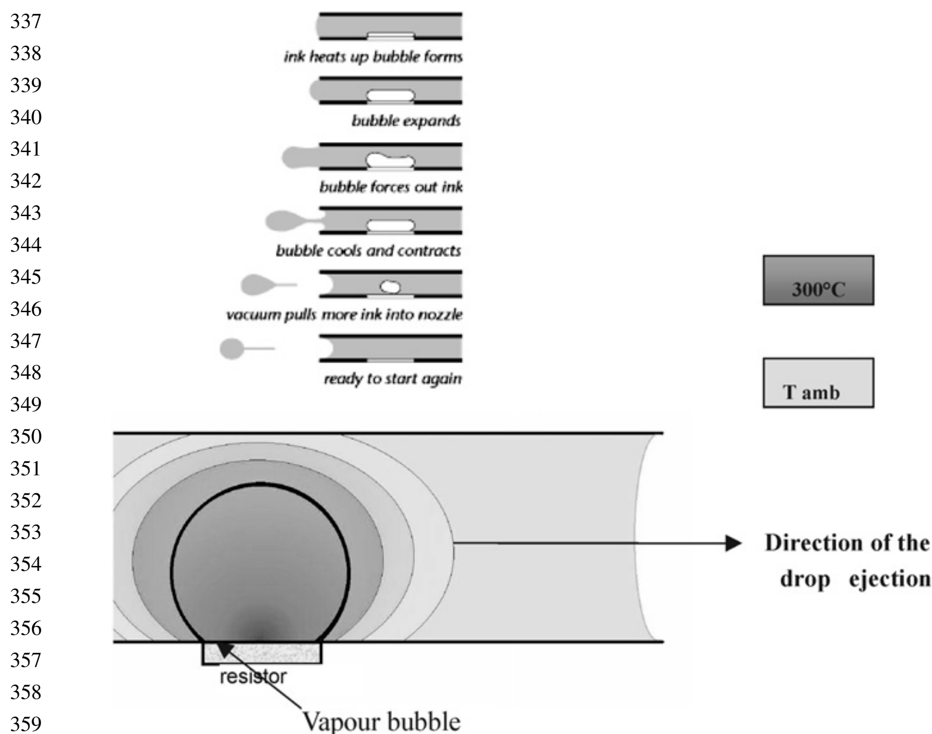


361 *Figure 3.* Schematic mechanism of the drop ejection through a thermal inkjet nozzle.
362 (*View this art in color at www.dekker.com.*)
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364 on the contrary, since it is recognized that thermal inkjet is a cheaper technology
365 with respect to the piezoelectric one, it is a promising candidate for economical,
366 reliable, precise, and controlled microdeposition of enzymes and, more in
367 general, other biological molecules.
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370 ## CONCLUSIONS

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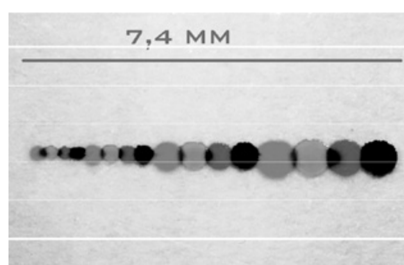
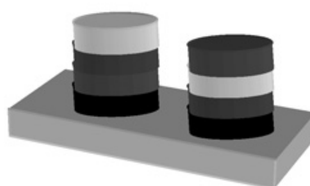
372 In the last two years strategies for formulating biological and electronic
373 water-based inks for inkjet technology, containing, respectively, different
374 enzymatic activities and conjugated polymers, opened the way for a wide
375 range of possibilities in the realization of printed biosensors.[8–10] Electrically
376 conducting polymers, in fact, have been found to be excellent materials for
377 immobilization of biomolecules, favoring the rapid transfer of the charge pro-
378 duced by the biochemical reaction to the electronic circuit.[12,14–16] These

systems represent the third generation biosensors, in which the enzymes transfer electrons directly to an electronically conducting polymer, without the presence of a third "mediator" molecule (which is needed in the second generation enzyme electrodes). In 2000 Sirringhaus et al.[17] have demonstrated a direct inkjet printing of complete transistor circuits, including via-hole interconnections based on solution-processed polymer conductors, insulators, and self-organizing semiconductors. This microdeposition technique permitted realization of integrated transistor circuits in a way far cheaper and easier than the traditional vacuum deposition and/or photolithographic patterning ones.

Widening this concept, in principle it is possible to exploit inkjet printing for ejecting microdots with different functionalities (for example biological or electronic), in very precise positions and on any surface. Moreover, the use of a commercial multicolor cartridge could allow the simultaneous deposition of microarrays containing multiple functionalities. The great potentiality of the inkjet technology in this field is hence linked to the possibility to design different matrices of dots in linear sequences, and even in one or more three dimensional pile structures (Fig. 4), eventually connected between them. This matrix




Linear sequence of dots



3-D pile structure of dots

***Figure 4.*** Two ways for assembling sequential series of different dots by mean of inkjet technology. (*View this art in color at www.dekker.com.*) EQ2

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could be constituted by various combinations of biological or electronic dots, properly connected and communicating with each other, which could be capable of performing a number of complex functionalities.

We propose that this concept takes the name of "biopolytronics," since it joins the already existing field of polytronics (polymeric electronics) and the one of biologically active molecules.

The next step in the progress of biopolytronics will consist then in the realization of the electronic communication between two different functionalities in sequence, constituted by a biological and an electronic ink, respectively, and work is currently underway in this line.[18] This achievement will be the first realizative step of the future biosensor technology, in which it will be possible to design a virtual, microsized biosensor, and immediately print it out on any surface using an inkjet multi-ink printer. Biopolytronics will be then the name of the game, which promises to be very exciting.

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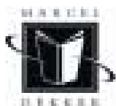

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