Proceedings of a C.I.S.B. Minisymposium – June 2010

Lab-on-chip system at "La Sapienza": When Thin and Thick Film Technologies meet Chemistry and Biological Applications

Domenico Caputo[1], Matteo Ceccarelli[1], Giampiero de Cesare[1], Augusto Nascetti[2], Riccardo Scipinotti[1]
[1]*Dept. of Electronic Engineering, "La Sapienza" University of Rome, Rome (Italy)*
[2]*Dept. of Aerospace and Astronautics Eng., "La Sapienza" University of Rome, Rome (Italy)*

Cesare Manetti[3], Andrea Leonelli[3], Francesca Costantini[3]
[3]*Dept. of Chemistry, "La Sapienza" University of Rome, Rome (Italy)*

Corrado Fanelli[4], Massimo Reverberi[4], Anna Adele Fabbri[4] ,Cristiano Bello[4], Alessandra Ricelli[5]
[4]*Dept. of Plant Biology, University "La Sapienza" University of Rome, Rome (Italy)*
[5]*Istituto di Chimica Biomolecolare, CNR, Rome (Italy)*

ABSTRACT

In this paper, we present a compact lab-on-chip system (LOC) fabricated on a conventional microscope glass slide using thin-film and thick-film technologies at "La Sapienza" University of Rome by the integration of know-how of researchers from Department of Electronic Engineering, Department of Aerospace and Astronautics Eng., Department of Chemistry and Department of Plant Biology.

It integrates a heating chamber, an electrowetting-based droplet handling system and hydrogenated amorphous silicon (a-Si:H) photosensor array for biomolecule detection. The heating chamber incorporates a thin metal film heater whose geometry has been optimized for uniform temperature distribution over a 1cm^2 area. An a-Si:H p-i-n junction integrated with the heater and biased with a forward current acts as temperature monitoring, achieving a sensitivity -3.3 mV/K with a linear behavior in the investigated range. The droplet-handling unit, relying on the electrowetting method, is designed to move the sample from the heating chamber to the sensor array. The unit includes a set of metal pads beneath a layer of PDMS that provides both the electric insulation of the electrodes and the hydrophobic surface needed by the electrowetting technique.

The detection unit has been applied to quantify Ochratoxin A (OTA) based on hydrogenated amorphous silicon (a-Si:H) sensors. 2 µl of acidified toluene containing OTA at different concentrations were spotted on the silica side of a High Performance Thin Layer Cromatography plate and aligned with a a-Si:H p-i-n photodiode deposited on the LOC. Results show a very good linearity between OTA concentration and the sensor photocurrent down to 0.1ng, showing that the presented system has the potential for a low cost system suitable for the early detection of toxins.

To extend the application of LOC to the analysis of real matrices the group are developing new chemical strategies, that are also presented in this paper.

Keywords: amorphous silicon, photosensor, mycotoxin detection, ochratoxin A.

1 INTRODUCTION

A variety of recent technological breakthroughs in molecular biology and microfabrication technology have made possible the development of lab-on-chip (LOC)

systems. The high integration level of the LOCs allows to accomplish complex chemical or bio-chemical functions of large analytical devices on a single sensor-like system with a fast response time, low sample consumption and on-site operation [1, 2]. The functional modules included in LOC systems are those capable of sample injection, reaction, separation and detection. In the framework of LOC systems the most promising are the DNA Chips (or DNA microarray) [3, 4], which are already commercially available. Interesting application of LOC were developed and are currently under study for different analytes.

In this paper, we present a compact lab-on-chip system (LOC) fabricated on a conventional microscope glass slide using thin- and thick-film technologies at "La Sapienza" University of Rome integrating the competences of researchers from different fields: electronics, chemistry and of biology. In particular, the system couples heating and movement of solution drops with quantitative on-chip detection performed by hydrogenated amorphous silicon (a-Si:H) photosensors directly deposited on the glass.

2 LAB-ON-CHIP SYSTEM CONCEPT

Three main functions of the sample-treatment chain have been implemented in our system as sketched in Figure 1. The pre-treatment unit provides sample heating by means of a thin film resistor acting as heater and an amorphous silicon diode acting as temperature sensor. The sample-handling unit moves the droplet samples along the lab-on-chip, while the detection unit quantifies the presence of specific biomolecules.

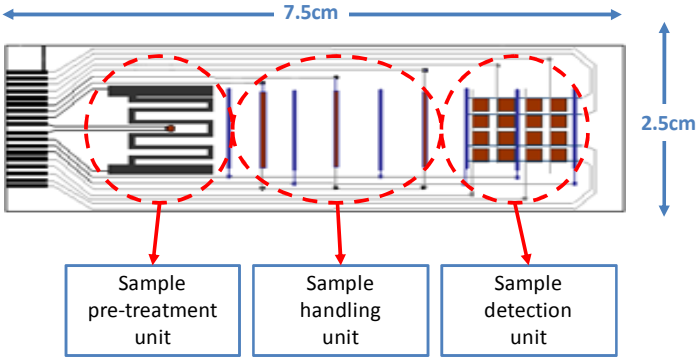

Figure 1. Structure of the Lab-on-Chip.

In this work, the three parts of the presented LOC have been studied as individual units taking into account the final project to integrate all of them in a single device. According to this specification, each functional block has been fabricated considering the requirements and compatibility of all the technological steps.

3 SAMPLE PRE-TREATMENT UNIT

In many applications pre-treatment involves a thermal cycling of the sample e.g. for DNA amplification by Polymerase Chain Reaction (PCR). In our system this function is implemented by a thin film heater, a temperature sensor and a PDMS chamber. In order to get a spatial-uniform temperature distribution over the whole active area the heater geometry has been designed using multiphysics finite element simulations (COMSOL Multiphysics), which couples the electrostatic problem and the heat transfer problem. We optimized the width and the spacing of the segments forming a serpentine-shaped resistor achieving a temperature distribution with uniformity better than 2% over the entire 1 cm^2 area. (solid line in Figure 2a). The heater has been fabricated with a 2000 Å thick Ti/W film resistor deposited by magnetron sputtering on the glass substrate. The active area of the heater is 1 cm^2. The measured resistivity is $3.8 \cdot 10^{-4}$ Ω/cm. In Figure 2b we report, as symbols, the measurements of the temperature distribution performed using a thermo-camera (AVIO NEOThermo TVS620P). The experiment has been performed applying a voltage of 30 V causing a maximum temperature of 90°C. The measured uniformity has been found to be better than 3% confirming the modeled results.

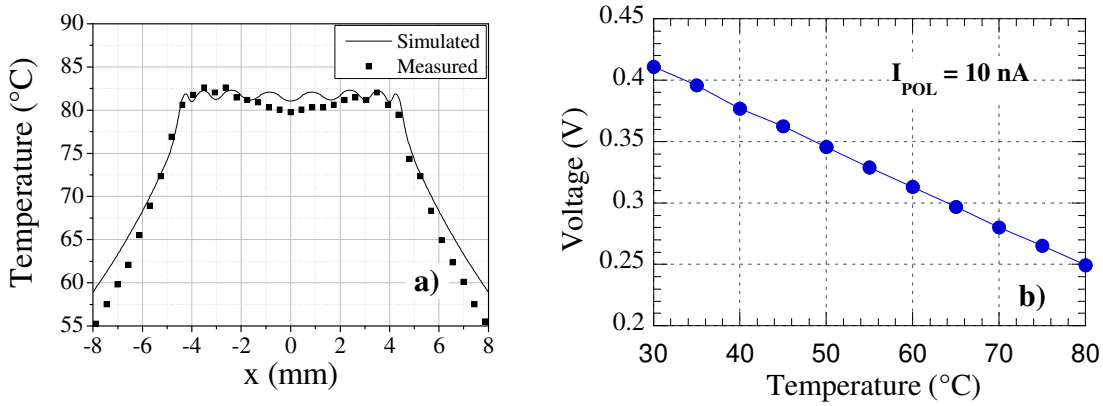

Figure 2. a) Simulated (line) and measured (symbols) temperature distribution of a thin-film resistor. b) Measured voltage across the a-Si:H diode, biased with 10nA constant current, as a function of temperature.

In order to integrate temperature sensing in the structure, the suitability of an amorphous silicon diode operating as temperature sensor has been investigated. For its fabrication, a 2000 Å thick film of Ti/W has been at first sputtered on the glass substrate as bottom contact of the device. In the complete LOC structure this electrode will to be deposited simultaneously with the thin film heater. The sensor is a n-type a-Si:H/i-type a-Si:H/p-type a-SiC:H (amorphous silicon carbide) stacked structure deposited by Plasma Enhanced Chemical Vapor Deposition (PECVD). The top contact has been ensured by a 2000 Å thick Ti/W metal layer. A square-shaped diode with area of 1mm^2 has been characterized performing current-voltage (I-V)

measurements at different temperatures ranging from 20°C up to 80°C with step of 5°C. We found that, in forward bias conditions, at constant bias current, the voltage across the diode is linearly dependent on the temperature. In particular, at 10 nA constant bias current, a sensitivity of -3.3 mV/K, as shown in Figure 2b, has been achieved. We have observed that this value does not significantly change with thicknesses of the a-Si:H layers. Therefore in order to reduce and to keep the technological steps as simple as possible, we deposited the a-Si:H diode with the same deposition parameters utilized for the UV photosensor described in the detection unit.

The confinement of the sample above the heater has been obtained fabricating a PolyDiMetylSiloxane (PDMS) chamber. The chamber has been bonded to the microscope slide by exposing the surfaces of both substrate and chamber to an oxygen plasma (40W, 3 minutes, 200mT, 100sccm). The water-tightness of the fabricated PDMS chamber has been verified introducing 100 µl of water inside the chamber and placing it on a hot plate heated at 100°C: after cooling down at room temperature the entire water sample has been removed from the camera confirming the absence of any leakage.

4 SAMPLE HANDLING UNIT

The handling unit is used to move DNA sample droplets from the heating chamber to the detection unit. The movement is achieved implementing the electrowetting-on-dielectric (EWOD) method. The EWOD relies on the possibility to change the contact angle of a liquid droplet in contact with a hydrophobic layer by means of an electric fields generated by an insulated control electrode, according to the Young-Lippmann equation [5, 6]. The typical EWOD structure described in literature [7] is constituted by an array of metal electrodes, an insulation layer (usually silicon nitride) that sustains the electric field, and an hydrophobic layer (typically PTFE, commonly known as Teflon).

In the proposed structure (see Figure 3a) the insulation and hydrophobic functions are performed by a single layer of PDMS, deposited on the glass substrate by spin coating. Even though, the suitability of PDMS as hydrophobic layer [8] and as insulation layer [9] respectively in EWOD devices has been already demonstrated, this is the first time, at our knowledge that it is used at the same time as insulation and hydrophobic layer in a EWOD device. As a first characterization, we deposited several PDMS layers with different spin rate and measured their morphological and electrical characteristics. We found that the sample deposited by spin coating at 6000 rpm for 30 sec and baked on hot plate at 165°C for 20 minutes in vacuum shows the best trade-off between smoothness of surface (which decreases with rpm) and thickness of the layer (which decreases with rpm). A better smoothness ensures a easier movement while a thinner sample allows the use a lower voltage to obtain the droplet movement. For this sample, a breakdown voltage greater than 20 MV/m and a contact angle around 117° have been achieved.

Starting from these results, we fabricated a EWOD structure consisting of an array of 1 mm^2 area squared Ti/W electrodes deposited by sputtering at 100°C on the glass substrate covered by a layer of PDMS deposited with the recipe reported above. The resulting PDMS thickness is 1 µm. The area of the electrode is enough to contain the 2µl water droplet typically

used in our biomolecular detection experiments [10, 11]. The gap between electrodes is 75 µm wide. This distance has been chosen as an average value between the data reported in literature [9].

We performed the experiment using a 2 µl of water and applying voltages, between adjacent electrodes, up to 200V. In Figure 3b it is visible the deformation of the drop caused by the applied voltage. Even though movement is achieved starting from 75Volts, only voltages above 100Volts demonstrated a reproducible movement of the droplet. No damage of the PDMS layer has been observed up to 200 Volts.

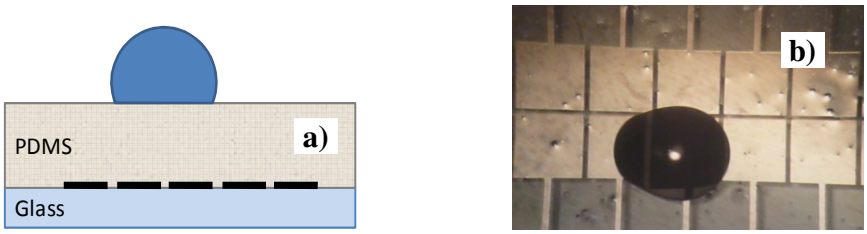

Figure 3. a) Cross section of the EWOD device. b) Frame showing the deformation of the droplet during the movement.

5 AN APPLICATION OF LOC: DETECTION OF OCHRATOXIN A

The suitability of the detection-unit to quantify the presence of biomolecules has been demonstrated revealing the naturally fluorescent Ochratoxin A (OTA). This toxin, produced by some fungi both in fields and during storage, can contaminate several food commodities as cereals, fruits and milk and are rather dangerous for the human being. A schematic view of the proposed system is reported in figure 4. One side of the glass substrate hosts the photosensor, while on the other side of the glass a solution, containing the analytes, is aligned with the sensor and properly confined. The ultra-violet (UV) radiation excites the biomolecule fluorescence that passes through the glass substrate and is absorbed by the sensor. The photocurrent generated inside the sensor is directly proportional to the amount of biomolecules. The thicknesses of the a-Si:H device have been designed to match the sensor spectral response with the emission spectra of OTA molecules and to keep the reverse saturation current as low as possible. From simulations by a numerical device program [12] and experimental results, we found that a good compromise between the above mentioned requirements is obtained using 100, 1500 and 500 Å thick p-doped, intrinsic and n-doped layers, respectively.
Results achieved with the system of figure 4 for detection of standard solutions of Ochratoxin A are reported in figure 5. The achieved limit of detection (LOD) of 0.1ng is comparable with those of expensive commercial equipment.

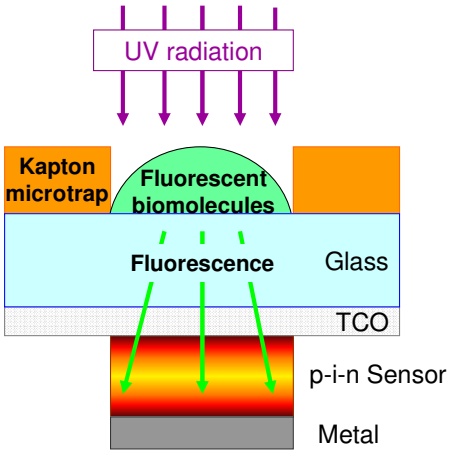

Figure 4. Scheme of the detection system

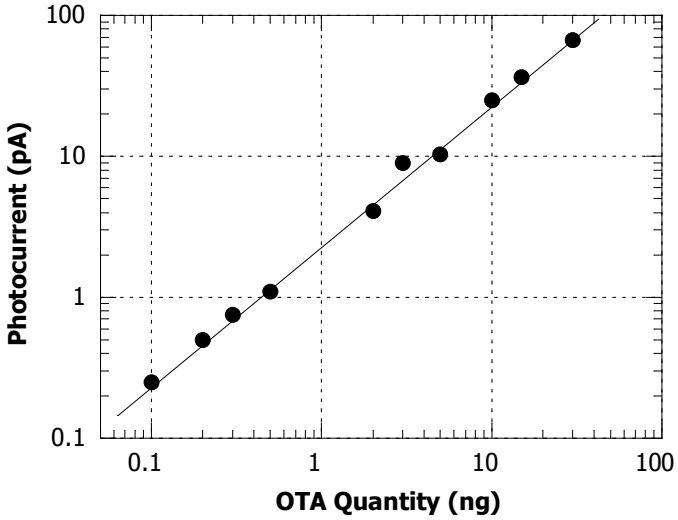

Figure 5. a-Si:H diode photocurrent vs OTA quantity

6 DETECTION OF OCHRATOXIN A IN REAL MATRICES

Results reported in section 5 refer to standard OTA and demonstrated the suitability of our system to detect OTA level well below of the law limit. In real matrix, however, the methodology for OTA analysis usually includes not only detection and quantification but also extraction, clean-up and separation. In particular, in order to analyze Ochratoxin A (OTA) in wine by lab-on-a-chip technology is necessary a preliminary separation step. This objective can be reached in different ways as described below with techniques originally developed by this work group.

6.1 Thin-layer chromatography

On the LOC can be easily integrated a thin-layer chromatography system, which allows separation and purification. Thin-layer chromatography is based on the polarity of the molecules. A spot of the mixture containing the molecules to be separated is deposited on an adsorbent layer (stationary phase), generally stratify on a laminated glass. Solvents (mobile phase) are dragged by capillary force through the stationary phase where the separation take place. The separation occurs due to the physical and chemical characteristic of both stationary and mobile phase. After separation of the complex mixture (wine), the analysis of the amount of OTA is performed using a fluorimetric analysis. As for the experiment presented above, the fluorescence signal of OTA is captured by the amorphous silicon photo-sensor.

6.2 Molecularly imprinted polymers as stationary phase for Ochratoxin A

Modern research in solid-phase extraction (SPE) is focused essentially on the fabrication of functional materials and device based on polymers. Functional polymer technology has attracted much attention over the past decade. In the MIP technique, polymerization of a functional monomers occurs in the presence of a print molecule (or template) and a cross-linker. Subsequently, the template is removed from the solid polymer, thereby producing cavities that are complementary in size, shape, and functional group orientations to the print molecule
Major advantage of MIPs in analytical method development include low material and labor costs, as well as their chemical and physical stability. The design of MIP materials for OTA is an interesting topic and other groups have already characterized the interaction between OTA and MIPs.

6.3 Polymer brushes as stationary phase for Ochratoxin A cromatography on a glass surface

The use of polymer matrices to perform chromatographic purification is widely described in literature, the techniques of purification in SPE cartridges containing polymer matrices are widely engaged in chemistry and biology. Our aim is to use these polymer brushes as stationary phase to isolate and analyze OTA when is present in the wine solution. Polymer brushes can be easily stratified on the glass surface of LOC, the physical and chemical characteristic can be tuned choosing different monomer type.

7 CONCLUSIONS

We have presented a Lab-on-Glass application to quantify OTA. The system is composed by three parts that have been studied, fabricated and characterized separately taking into account the requirements for a future integration on the same glass substrate. A thin film heater with an integrated p-i-n a-Si:H diode constitute the pre-treatment unit. An array of sputtered Ti/W electrodes allows to move droplets of the solution containing the DNA from the heating chamber to the detection section by using EWOD methods. a-Si:H UV photosensor is used to detect the analytes. To extend the application of LOC to the analysis of real matrices the group is developing new chemical strategies, that have been presented in the paper.

8 REFERENCES

1. Kopf-Sill AR (2002), Lab Chip 2:42N–47N.
2. Manz, A.; Graber, N.; Widmer, H. M. Sens. Actuators 1990, B1, 244-248.
3. S.K. Moore, IEEE Spectrum, pp. 54-60, (2001)
4. Eric T Lagally and Richard A Mathies, J. Phys. D: Appl. Phys. 37 (2004) R245–R261

5. B. Berge, C. R. Acad. Sci. Ser. II: Mec., Phys., Chim., Sci. Terre Univers, 1993, 317, 157–163.
6. M. Vallet, B. Berge and L. Vovelle, Polymer, 1996, 37, 2456–2470.
7. Roland Baviere, Jerome Boutet, Yves Fouillet, Microfluid Nanofluid (2008) 4:287–294.
8. Ding, Hui-Jiang; Liu, Kan; Zhao, Li-Bo; Zeng, Qian; Guo, Zhi-Xiao; Guo, Feng; Zhao, Xing-Zhong, Bioinformatics and Biomedical Engineering, 2007. ICBBE 2007. The 1st International Conference on Volume , Issue , 6-8 July 2007 Page(s):1317-1320.
9. Mohamed Abdelgawad and Aaron R. Wheeler, Adv. Mater, 1007, 19, 133-137.
10. D. Caputo, G. de Cesare, A. Nascetti, R. Negri, R. Scipinotti, IEEE Sensors Journal, vol. 7 (9), pp. 1274-1280 (2007)
11. D. Caputo G. de Cesare A. Nascetti M. Tucci, IEEE Trans. of Electron Device, vol.55(1), p. 452-456 (2008).
12. D. Caputo; U. Forghieri; F. Palma, J. Appl. Phys. Vol. 82, pp. 733-741, (1997).