Bioelectrical and metabolic activity recordings by means of organic field effect transistors

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***Abstract*— The introduction, in the early 70's, of the Ion Sensitive Field Effect Transistor (ISFET), completely revolutionized the biosensing field thanks to its versatility and the reliability of its transduction principle. Since then, a lot of different ISFET-based biosensors have been studied and realized for a great number of applications. Among them, a fast growing field of application is the electrophysiological field, in which ISFET-based sensors are employed for the detection of several parameters of electroactive cells, such as their electrical and metabolic activity. Here an innovative system based on a particular kind of Organic Thin Film Transistor (OTFT), called Organic Charge Modulated FET (OCMFET), is presented as a flexible, transparent, and low cost alternative to common ISFETs for the transduction of the electrical and metabolic activity of electrogenic cells. The exploitation of organic electronics in the electrophysiological field may bring several advantages over the existing techniques, thus introducing a novel paradigm in the way in which we interface living systems.**

Keywords—in-vitro electrophysiology; organic field effect transistors; pH monitoring

I. INTRODUCTION

To date, two kinds of electronic devices dominate the electrophysiological field: Micro Electrode arrays (MEAs) and Field Effect Transistor based devices (FETs). Although these devices are widely and successfully employed, they suffer from several drawbacks, such as the high costs associated to their fabrication and the intrinsic rigidity of the materials. Another problem that affect their use as (bio)sensors is the need of a reference electrode immersed in the measuring environment. This represents undoubtedly one of the most important issues in biosensing since reference electrodes are usually not easy to integrate in CMOS technology, and they are typically bulky, thus limiting the miniaturization of the device.

The employment of organic electronics, however, may bring several advantages in the biosensing field, thanks to the low costs of fabrication techniques and the almost countless number of different flexible, lightweight, transparent, and biocompatible materials. In the last decade, a lot of effort has been put toward the realization of different kind of Organic FET-based chemical and bio-sensors with a multitude of working principles as Ion Sensitive OFETs [1], OECTs [2] and Electrolyte-Gated OFETs [3]. The key issues related to these devices are: (i) the presence of a reference electrode (usually an Ag/AgCl electrode), that limits the miniaturization of the device (as for "classic" inorganic devices); (ii) the degradation of the semiconductor layer due to the fact that the organic material is in direct contact with the liquid environment. A very interesting organic device, the Organic Charge Modulated FET (OCMFET) [4], is able to overcome the above-mentioned limitations thanks to the presence of an elongated floating gate and a sensing mechanism that allows separating the semiconductor layer and the sensing area. Additionally, a second gate, called control gate acts as a reference for the device and eliminates the need of an external reference electrode. In the recent past, different versions of the OCMFET have been already successfully tested as pH sensors [5] and as DNA hybridization sensors [6].

In this work, we will focus on interfacing an OCMFET device specifically tailored for *in-vitro* electrophysiological applications with living cells cultures and we shall propose an innovative approach for the simultaneous detection of both the electrical and the metabolic activity of living cells.

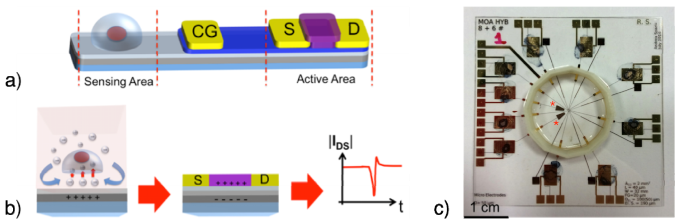

Figure 1 a) Cross section of an OCMFET device. CG is the control gate while S and D are the source and the drain contact respectively. b) Working mechanism of the OCMFET for cell electrical activity sensing. The ions displacement during an action potential causes a charge redistribution inside the floating gate metal which in turn causes a variation of the threshold voltage of the transistor and a modulation of its output current. c) Top view of an MOA with 8 OCMFETs dedicated to cell electrical activity detection, 2 OCMFETs for metabolic activity monitoring (red asterisks), and 6 passive microelectrodes used as internal controls. The sensing area is delimited by a plastic ring and is separated from the semiconductor layer.

II. THE ORGANIC CHARGE MODULATED FET

A. OCMFET structure

The organic charge modulated FET (OCMFET) is an ultra-low voltage floating gate Organic Thin Film Transistor (OTFT) in a bottom-gate/bottom-contact configuration biased through a control capacitor. The floating gate is elongated, thus allowing keeping the sensing area, i.e. the exposed part of the floating gate, far from the transistor area. This configuration is crucial to preserve the organic semiconductor layer, which is constituted by a thin layer of 6,13-Bis(triisopropylsilylethynyl) pentacene (TIPS) deposited by means of drop casting. Moreover, this device does not need any external reference electrode thanks to the presence of the control gate, through which is possible to set the transistor working point. A cross section of the OCMFET is shown in figure 1a.

The OCMFET was fabricated onto a 250 µm polyethylene terephthalate (PET) substrate, a flexible, biocompatible, transparent, and low cost material. The floating gate was obtained from a thermally evaporated titanium layer, which was patterned by means of a standard photolithographic process. An ultra-thin, hybrid organic/inorganic double-layer insulator, composed by few nanometers of native metal oxide and, few tenths of Parylene C layer, was employed as gate dielectric in order to obtain the low-voltage behavior (similarly to what previously reported in [7]).

B. Working principle

The OCMFET working mechanism is related to a variation of the threshold voltage induced by an external charge present onto the metal oxide of the exposed part of the floating gate. This charge can be either immobilized onto the sensing area or time-varying. The threshold voltage variation is described by the following equation:

$$\Delta V_{TH} = -\frac{Q_{SENSE}}{C_{CG}+C_{SF}+C_{DF}} \qquad (1)$$

where Q_{SENSE} is the charge to be sensed, C_{CG} is the control gate capacitance, and C_{SF} and C_{DF} are the parasitic capacitances that originate from the overlap between source and floating gate and drain and floating gate, respectively [8].

In the case of cell electrical activity, the charge to be sensed is a time-varying charge that reflects the ions movement through the portion of the cell membrane adhering onto the sensing area during an action potential. The threshold voltage fluctuation can be read as a variation of the transistor's output current I_{DS}, as depicted in figure 1b. In the specific case of metabolic activity, the pH variations are measured as the varying charge present onto the sensing area related to the slow process of protonation or de-protonation of superficial silanol sites of the oxide due to the acidification process (starting from a physiologic condition of 7.4±0.1 pH) caused by cells metabolism. The proposed device is an OCMFET array and it is called micro OCMFET array (MOA) and it consists of up to 16 OCMFET. The presence of a plurality of organic transistors is needed to perform multisite recordings. A complete MOA with 8 OCMFETs dedicated to electrical activity transduction, 2 OCMFETs dedicated to pH monitoring, and 6 passive microelectrodes used as internal controls is shown in figure 1c.

III. EXPERIMENTAL RESULTS

A. Cell electrical activity

The OCMFET array has been tested with cardiomyocytes cultures from rat embryos (E18). Figure 2a shows an healty cardiomyocyte culture adhering onto the sensing area of a MOA device, while in figure 2b few seconds of spontaneous activity of a cardiomyocyte culture (8 Days In Vitro, 8 DIV) are shown. The electrical readout was performed using a multichannel readout electronics specifically designed and fabricated in order to filter and amplify the output current of the sensor. The electrophysiological measurements was performed at 37 °C in a controlled atmosphere (5% CO2 and 95% of humidity).

B. pH sensitivity

The OCMFET device was also preliminary tested as pH sensor in order to demonstrate the possibility to sense the pH variation induced by the metabolic activity of cell cultures. No additional layer was used onto the sensing area to enhance the sensitivity to pH changes in solution since the protonation of the metal oxide onto the surface of the sensing area was found sufficient to induce a shift of the threshold voltage of the transistor. In order to test the pH variation detection capabilities, the sensing area of the device was exposed to three different solutions with different pH values (namely 4, 7 and 10) and a static characterization of the OCMFET was performed for each solution. In figure 3a the input characteristic of an ultra-low voltage OCMFET for different pH values is shown, together with the calibration curve of the sensor (Figure 3b).

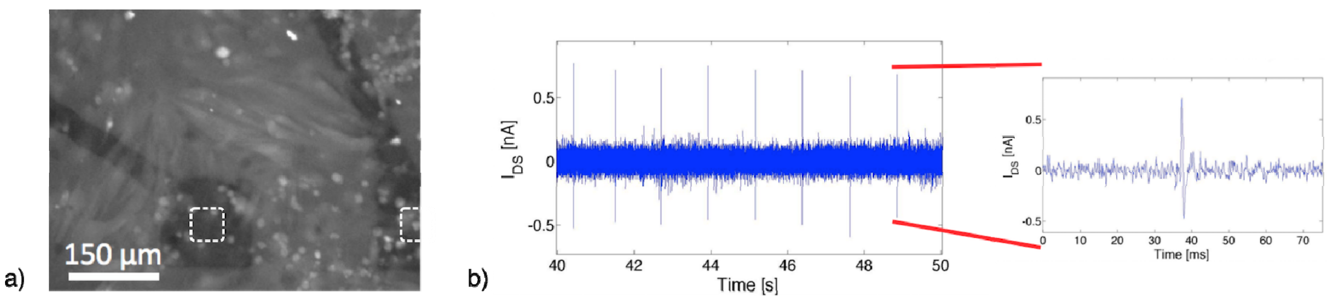

Figure 2 Cell cultures and experimental results. a) Magnification of the sensing area of an OCMFET. A confluent culture of rat cardiomyocytes maintained 8 days in vitro (8 DIV) is adhering on the surface. The opening in the passivation layer (white squares) allows obtaining sensing areas dimensions of 50 µm X 50 µm. b) Raw data relative to ten seconds of spontaneous activity of the same rat cardiomyocyte culture recorded with an OCMFET. Each peak in the trace represents an extracellular cardiac field potential. b) (inset) Magnification of a cardiac myocyte single signal recorded with an OCMFET device.

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Figure 1. Cross section of the device.

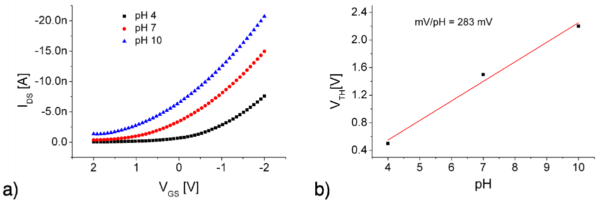

Figure 3 OCMFET response to pH variations. a) Transfer characteristic of an OCMFET device taken at different pH values. b) Calibration curve of the sensor.

IV. Conclusions

A floating gate OTFT was presented and characterized as a sensor for electrophysiological and metabolic applications. The proposed device, called organic charge modulated FET (OCMFET), is flexible, transparent, and low cost, and its ability to detect the electrical activity of electrogenic cells without the need of any external reference electrode has been thoroughly demonstrated using rat cardiomyocytes cultures. The possibility to exploit the OCMFET's interesting properties for the detection of pH variations related to the cells metabolism was also investigated, and sensitivities up to 283 mV/pH are here reported. By considering the capability of the device of transducing both relatively fast electrical signals and slow or quasi static metabolic signals, OCMFET based systems are good candidates for a new generation of low cost, flexible, and transparent organic devices for *in-vivo* and *in-vitro* electrophysiological applications and for neuro-electronic interfaces.

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