From MEAs to MOAs: The Next Generation of Bioelectronic Interfaces for Neuronal Cultures

Andrea Spanu, Mariateresa Tedesco, Sergio Martinoia, and Annalisa Bonfiglio

Abstract Since their introduction in the early 1970s, microelectrode arrays (MEAs) have been dominating the electrophysiology market thanks to their reliability, extreme robustness, and usability. Over the past 40 years, silicon technology has also played a role in the advancement of the field, and CMOS-based in vitro and in vivo systems are now able to achieve unprecedented spatial resolutions, giving the possibility to unveil hidden behavior of cellular aggregates down to the subcellular level. However, both the MEAs and silicon-based electronic devices present unavoidable problems such as their expensiveness, the usual rigidity of the employed materials, and the need of an (usually bulky) external reference electrode. Possible interesting alternatives to these incredibly useful devices unexpectedly lie in the field of organic electronics, thanks to the fast-growing pace of improvement that this discipline has undergone in the last 10–15 years. In this chapter, a particular organic transistor called organic charge-modulated field-effect transistor (OCMFET) will be presented as a promising bio–electronic interface, and a complete description of its employment as a detector of cellular electrical activity and as an ultrasensitive pH sensor will be provided, together with the discussion about the possibility of using such a device as an innovative multisensing tool for both electrophysiology and (neuro)pharmacology.

Keywords OCMFETs · MEAs · Cell electrical activity monitoring · pH sensing · Pharmacology

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1 Introduction

The beginning of the "microelectrodes array era" can be traced back to the first half of the 1970s, thanks to the seminal paper of Thomas (Thomas et al. 1972), which followed more than two decades of studies on single unit metal electrodes for biological applications (Hubel 1957; Verzeano et al. 1960; Robinson 1968; Wise et al. 1970). This work introduced the idea that it was possible, and somehow convenient, eavesdropping the "cellular chat" from the outside of the cell, instead of being confined by the cell membrane borders, by using a very simple tiny metal electrode. This change of paradigm virtually gave rise to the modern neuroscience field as we know it, allowing for the study of cells aggregates over long periods with a non-destructive approach, and opening up a whole world of new possibilities in the understanding of the central nervous system, and promoting the advent of disciplines such as the brain–machine interface (BMI) field. The seed rapidly spread, and in the following decades an increasing number of MEA-based systems have been designed and implemented, with an escalation of new materials, fabrication techniques, and novel applications (Gross et al. 1977; Rousche et al. 2001; Takeuchi et al. 2004; Blau et al. 2011; Sessolo et al. 2013).

Indeed, the 70s of the twentieth century turned out to be a very important decade for electrophysiology. Besides the advent of MEAs, another important device saw the light in the 1970, namely the ion sensitive FET (ISFET). The very first example of ISFET was introduced by Piet Bergveld as a neurophysiological tool (Bergveld 1970, 1972), and few years later another version of this device, called OSFET, was further optimized for the detection of bioelectrical signals in vitro (Bergveld et al. 1976). The idea of using ISFET-like electronics fascinated the scientific community, and the great effort put in the optimization of the device culminated 20 years later with the work of Fromherz (Fromherz et al. 1991; Weis et al. 1996; Vassanelli and Fromherz 1997; Stett et al. 1997), whose model of neuron–FET interface in vitro is still widely accepted and used in the development of innovative silicon-based systems (Berdondini et al. 2005, 2009; Krause 2000; Ecken et al. 2003; Meyburg et al. 2006; Viswam et al. 2016; Lopez et al. 2018).

Despite their widespread use and the fact that they have definitely conquered the electrophysiological field, the drawbacks associated to the MEA and the ISFET technology are patent, such as the problems associated with the high cost of fabrication, the rigidity (usually) associated to the materials, and the presence of a (again, usually) bulky external reference electrode.

In the last years, the growing need for low-cost and possibly disposable in vitro electrophysiological tools (aiming at the reduction of animal-demanding in vivo experiments) put the organic (bio)electronics field in the limelight. Organic electronics, in fact, represents an interesting alternative in all those applications where inexpensiveness, mechanical compliance, and biocompatibility are required. To date, among the few organic devices that have been employed as bio–electronic interfaces, the organic electrochemical transistor is undoubtedly the more studied. This device can be operated with ultralow voltages and has been able to

reliably detect neuronal activity in vivo, outperforming passive microelectrodes (Khodagholy et al. 2013, 2015). Lately, electrolyte gated organic FETs (EGOFETs) have also been extensively studied and employed as sensor and biosensors (Kergoat et al. 2012; Casalini et al. 2013; Schmoltner et al. 2013), and recently, an attempt to introduce this interesting tool in the cellular domain has been done (Zhang et al. 2017). Although conceptually interesting, the work is at a very early stage, and the actual application of this device in electrophysiology has yet to come. Despite their potentials, OECTs and EGOFETs present drawbacks such as the need of an external reference electrode and the direct exposure of the semiconductor layer to the harsh liquid environment where the sensing takes place, with the former issue not allowing the single device addressability in an array configuration and the latter making these devices not suitable for long-term biological applications.

Besides OECTs and EGOFETs, another interesting organic device, named organic charge-modulated FET (OCMFET), has been recently developed and successfully employed for the detection of electrical activity in vitro. In the following sections, all the different aspects of the OCMFET, from the working principle to future developments, will be presented with the intent of giving an overview of the possible advantages that this versatile organic transistor may bring to electrophysiology and pharmacology.

2 The Organic Charge-Modulated Field-Effect Transistor

The OCMFET is a floating gate organic thin film transistor (OTFT) in a bottom-gate/bottom-contact configuration. The device is gated through an additional contact called control gate, and it can be converted into a high-sensitive charge transducer by exposing the final part of the elongated floating gate to the measurement environment. In fact, the presence of a charge (which is capacitively coupled to the floating gate through an insulating spacer) onto the sensing area induces a shift of the threshold voltage V_{TH} of the device, which can be read out as a modulation of the output current of the transistor. In Fig. 1a, b, the structure and the electrical characteristics of an OCMFET device are shown.

The concept of the device (which was initially developed in CMOS technology) has been proposed by Barbaro et al. in 2006 (Barbaro et al. 2006); its working principle can be explained starting from the expression of the charge Q_{TOT} in the floating gate, which can be estimated taking into account the different voltage contributions in the device according to Gauss equation:

$$Q_{TOT} = C_{CG}\,(V_{FG} - V_{CG}) + C_{DF}\,(V_{FG} - V_{D}) + C_{SF}\,(V_{FG} - V_{S}) \qquad (1)$$

where C_{CG}, C_{DF}, and C_{SF} are, respectively, the control capacitance and the parasitic capacitances related to the overlap between drain, source, and the floating gate; V_{CG}, V_{D}, and V_{S} are the voltages applied to control capacitor, drain, and source

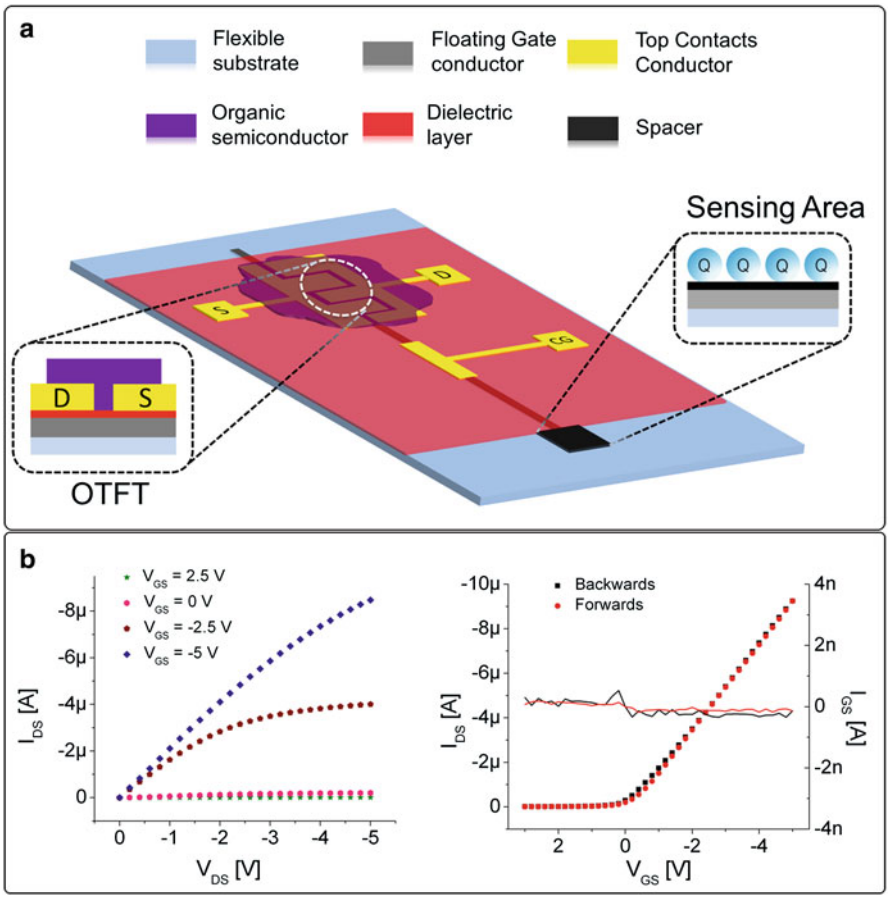

Fig. 1 (**a**) Representation of an OCMFET device. The OCMFET is a floating gate OTFT with a control gate that is needed to set the transistor's working point. The device can be employed as a charge sensor by exposing the final part of the floating gate (called sensing area) to the measurement environment. (**b**) Output and input characteristic of a low-voltage OCMFET biased through the control gate

respectively and V_{FG} is the actual floating gate voltage. This last parameter can be written as:

$$V_{FG} = \frac{C_{CG}}{C_{TOT}} V_G + \frac{C_{DF}}{C_{TOT}} V_D + \frac{C_{SF}}{C_{TOT}} V_S + \frac{Q_{TOT}}{C_{TOT}} \tag{2}$$

where $C_{TOT} = C_{CG} + C_{DF} + C_{SF}$.

If a charge Q_{SENSE} is present on top of the sensing area (and under the hypothesis of perfect charge induction), Q_{TOT} can be written as $Q_0 - Q_{SENSE}$, being Q_0 a constant amount of charge incorporated in the floating gate during the fabrication process, and Q_{SENSE} the charge present onto the sensing area. When the spacer

is thinner than the gate dielectric and Q_0 is negligible, the last equation can be approximated as

$$V_{FG} \approx V_G - \frac{Q_{SENSE}}{C_{TOT}} \tag{3}$$

Therefore, the floating gate voltage is linearly related to the amount of charge capacitively coupled to the sensing area. The charge variation ΔQ_{SENSE} can be transduced as a corresponding variation of the transistor's threshold voltage:

$$\Delta V_{TH} = -\frac{\Delta Q_{SENSE}}{C_{TOT}} \tag{4}$$

Unlike the other structures mentioned in this chapter, namely MEAs, OFETs, ISFET-like devices, OECTs, and EGOFETs, the OCMFET does not need any external reference electrode while operated as a sensor; this feature is particularly important when dealing with in vitro (but also in vivo) applications, since such an additional electrical contact usually represents one of the main obstacles to the device portability and miniaturization. Another important feature of the OCMFET is that its sensing mechanism only depends on the nature of the surface of the sensing area, being the organic transistor only used as an amplifier. This physical decoupling of transistor and sensing area brings several advantages in terms of device functionality and stability, allowing for the organic semiconductor to be encapsulated, thus drastically improving the durability of the system. The remarkable versatility of this technological choice has been thoroughly demonstrated during the past years, during which several different sensors have been designed and successfully tested, such as for example DNA hybridization sensors, pressure sensors, and pH sensors (Caboni et al. 2009; Lai et al. 2013a; Spanu et al. 2016).

3 The Micro OCMFET Array: Towards Multisensing Electrophysiological Tools Based on Organic Transistors

As previously highlighted, the OCMFET is a very convenient approach in all those applications where the detection of low charge variations in a liquid environment is involved, such as monitoring the electrical activity of living cells for pharmacology, (neuro)rehabilitation, BMIs, and computational neuroscience. Besides the prementioned features (i.e., the absence of an external reference electrode and the elongated shape of the floating gate, which allows separating the organic semiconductor and the sensing area), other interesting features are its high charge sensitivity, the possibility to be operated at low voltages (Cosseddu et al. 2012), and its relatively high cutoff frequency (up to 100 kHz), due to a high-k–low-k composite dielectric layer (Lai et al. 2013b). Those features make the OCMFET a good candidate for the design of novel electrophysiological/pharmacological tools that can be both

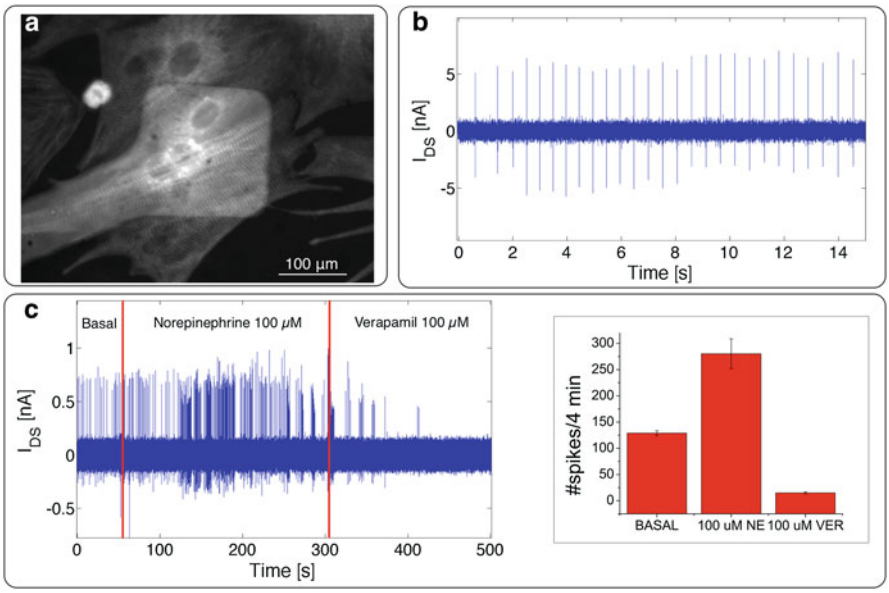

Fig. 2 OCMFET for electrophysiological applications: validation with primary cardiac myocytes from rat embryos. (**a**) Healthy cardiomyocytes onto a sensing area. The culture has been fixed after the recording session and immunostained for the sarcomeric protein Tropomyosin. (**b**) Basal activity of a cardiomyocytes culture maintained 8 days in vitro. (**c**) Chemical modulation of the culture's activity. The basal activity was accelerated using 100 µM of norepinephrine and then suppressed with 100 µM of verapamil. (**c**) (Inset), Beating frequency modulation (statistics on five OCMFETs fabricated within the same MOA) (Spanu et al. 2015; Spanu 2016). Copyright 2015, Nature Publishing Group

referenceless and low cost, thus potentially having the capability to compete with MEA and ISFET technologies. In order to meet the specific requirements of the electrophysiological application, a device called Micro OCMFET Array (MOA) has been recently designed and fabricated, and its capability of transducing bioelectrical signals has been thoroughly investigated (Spanu et al. 2015; Spanu 2016). Primary cardiomyocytes cultures from rat embryos have been chosen as the cellular model for the device sensitivity estimation, due to the optimal covering of the sensing areas that they provide and to their "pace-maker" electrical activity in vitro, which makes the recorded signals highly reproducible and predictable (indeed, very important aspects when dealing with the validation of a new sensor). In Fig. 2a healthy cardiomyocytes cultured onto the sensing area of an OCMFET are shown. The MOA turned out to be capable of reliably monitoring the activity of this kind of culture in both basal conditions and upon chemical stimulation, as reported in Fig. 2b, c.

Using the set of equations previously derived, it is possible to speculate on the sensing capability of the OCMFET. By considering the experimental recordings performed with several devices, the I_{DS} variation associated to a cardiac action potential ranges from hundreds of pA to few nA. By considering an average I_{DS}

variation of 1 nA and representative values of the electronic parameters of the device (a transconductance g_m of 300 pA/mV and the sum of the capacitances C_{TOT} of 100 pF), it is possible to estimate the corresponding charge variation that occurs onto (or in proximity to) the sensing area. For an OCMFET polarized in its saturation region (with $V_{GS} = V_{DS} = -1$ V), it is possible to estimate the relative variation ΔV_{FG} of the floating gate potential induced by the ΔI_{DS}:

$$\Delta V_{FG} = \frac{\Delta I_{DS}}{g_m} \tag{5}$$

As described in Eq. (5), ΔV_{FG} depends linearly on the charge Q_{SENSE}; by considering this charge as being completely associated to the charge displacement occurring on the sensing area during an action potential (Spanu 2016), it is possible to obtain a value of about 0.3 pC. By assuming that this variation is entirely due to the ions crossing the cell membrane during the upstroke of an action potential and by considering a typical membrane capacitance and a cardiac intracellular action potential amplitude ($C_{mem} = 1$ μF/cm^2 and $V_{INTRA} = 120$ mV respectively) it is possible to estimate the corresponding effective area A_{eff} of the cell membrane that faces the sensing area (thus where the charge variation occurs):

$$Q_{SENSE} = C \cdot V_{INTRA} \tag{6}$$

If $C = C_{mem} \cdot A_{eff}$, A_{eff} can be estimated, obtaining a value of 250 μm^2, which is consistent with the adhesion area of a cardiomyocyte soma, thus confirming the plausibility of the proposed transduction principle.

Following this important validation step, the MOA has been preliminary tested with neuronal cultures, a definitely trickier cellular model in terms of signal amplitude and predictability. The stability of the MOA device with neurons was therefore evaluated by using post-natal hippocampal neuronal cultures. In Fig. 3a, a healthy culture of hippocampal neurons cultured onto a MOA device for 21 days is shown (yellow stars indicate some well spread neuronal somata), thus demonstrating the suitability of the system for long-term in vitro neuronal applications. To test the device stability over time (and thus ensuring the feasibility of the device to be used in long term experiments) an OCMFET has been kept inside an incubator (37 °C, 95% of humidity, and 5% of CO_2) for 50 day and the variation of a typical parameter (the charge carriers' mobility) has been monitored. As shown in Fig. 3b, the device remained stable during the whole period. Interestingly enough, as can be noticed in Fig. 3c, d, the OCMFET turned out to be able to reliably monitor both the basal and the drug-mediated activity of such a culture, thus demonstrating the possibility of using the proposed device as a neuropharmacological tool.

Among the various interesting parameters when dealing with cell cultures, one of the most studied in the past 15 years is the metabolic activity. In fact, this aspect of cells behavior is particularly prone to change in response to various external stimuli and drugs, making it a particularly accurate way of assessing cellular viability. One possible method to monitor the cellular metabolism is measuring the

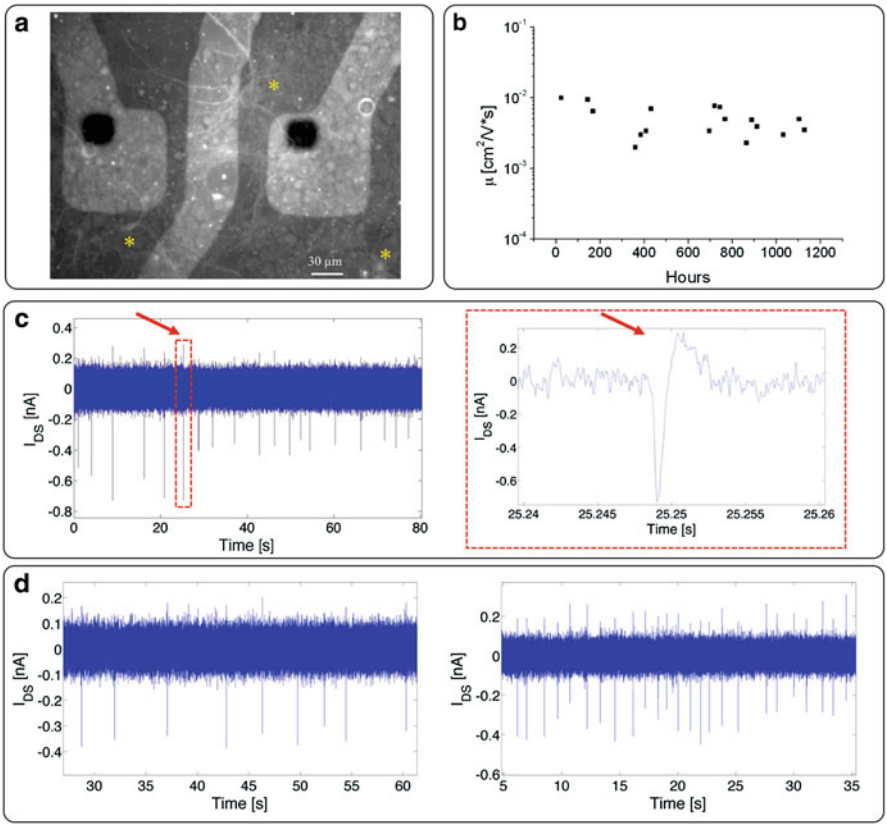

Fig. 3 (**a**) A healthy neuronal culture (hippocampal neurons, 21 DIV), fixed after the recording session. (**b**) Stability assessment of an OCMFET. The device mobility turned out to be stable over a period of 50 days inside an incubator (37 °C, 95% of humidity, and 5% of CO_2). (**c**) Example of hippocampal basal activity (21 DIV) measured with an OCMFET device. (**c**) (inset) Shape of a single hippocampal action potential. (**d**) Chemical modulation of neuronal activity. The basal activity (left) has been modulated by means of the addition of a mixture of drugs (right), namely 25 µM of BIC and 50 µM of 4AP. Copyright 2016, Springer

medium acidification caused by the extracellular accumulation of acidic byproducts (Hynes et al. 2009). Moreover, cells are highly sensitive to local pH variations, which can induce a modification of their physiological state, thus constituting a very important parameter to consider during whatsoever electrophysiological and/or pharmacological experiment.

In the recent past, a number of different approaches have been employed in order to meet the important requirement of reliably monitoring cells metabolism (Hafner 2000; Martinoia et al. 2001; Baumann et al. 1999; Yu et al. 2009). Despite the effort, the goal of having a multisensing platform for such an application is yet to be achieved, and this is mainly due to the complexity and high cost of the existing systems.

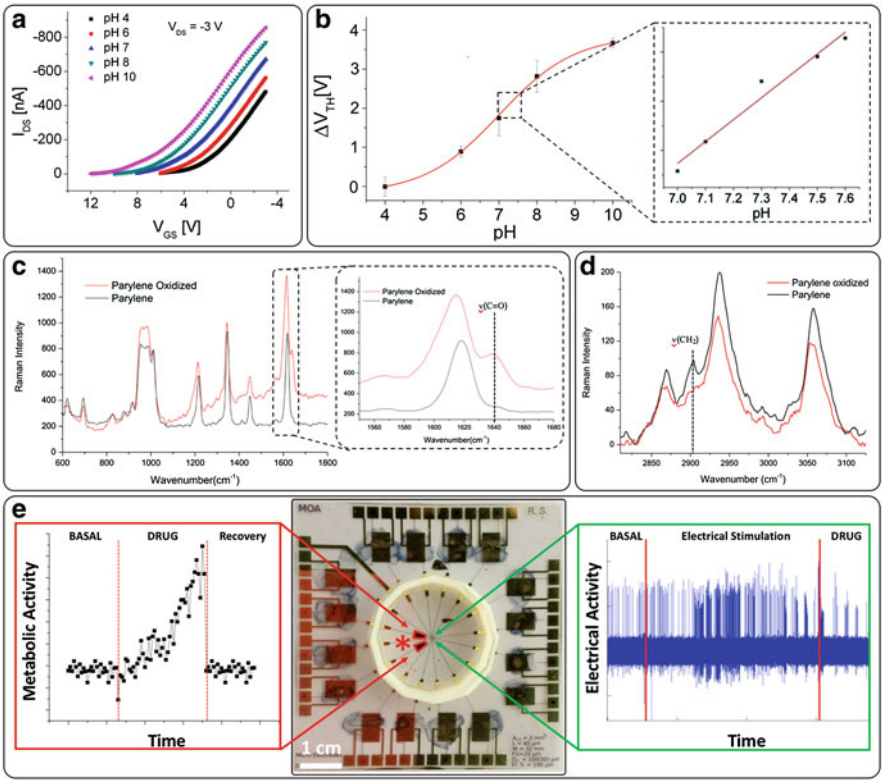

Fig. 4 Characterization of a pH-sensitive OCMFET. (**a**) Transfer characteristics performed while the sensing area is exposed to buffer solutions at different pH values. It is noticeable the gradual shift of the transistor's threshold voltage towards more positive values as the pH increases. (**b**) V_{TH} VS pH calibration curve. The device shows a sigmoidal behavior and has its linear region between pH 6 and pH 8. (**c**) Sensing layer characterization. Raman spectra of pristine Parylene C (black) and of the same Parylene C membrane after oxygen plasma exposure (red). The additional band at 1640 cm^{-1} is related to the vibration of the C=O stretching of the carboxyl group, while the vibration at 2900 cm^{-1} (**d**), which is related to the CH_2 stretching, decreased after the oxidation process with the related increase of the band at 1640 cm^{-1} (Spanu et al. 2017). Copyright 2017, Elsevier. (**e**) Example of a possible final design of a multisensing MOA for cellular applications containing pH-sensitive devices for metabolic activity monitoring (red) and channels for the detection of neuronal electrical activity (green)

To the aim of developing a highly efficient and possibly disposable tool for high-throughput in vitro toxicity assays and pharmacology (and thanks to its remarkable versatility), the OCMFET has been turned into an ultrasensitive pH sensor by using a simple sensing area functionalization, being the key element of the proposed approach a simple pH-sensitive membrane, consisting in a Parylene C thin layer exposed to oxygen plasma. As shown in Fig. 4a, b, such an OCMFET turned out to be a very sensitive pH sensor, thanks to the intrinsic charge amplification

given by the peculiar double-gated structure of the device (Spanu et al. 2017). As demonstrated by the Raman spectra shown in Fig. 4c, d, the plasma-activated Parylene C membrane undergoes a surface modification consisting in the exposure of superficial groups (mainly carboxyl groups), which can be protonated or deprotonated depending on the pH. The transduction principle, as previously explained, is related to a variation of the transistor threshold voltage induced by the (pH-dependent) charge immobilized onto the sensing area.

As proposed in Fig. 4e, these super-Nernstian and referenceless pH sensors can be easily integrated in a MOA device together with the OCMFETs for cell electrical activity monitoring, thus opening up the interesting possibilities for the fabrication of innovative low-cost and referenceless multisensing devices that could be able to monitor not only the electrical activity but also the metabolism of cell aggregates in vitro.

4 Conclusions and Future Prospects

The concept of OCMFET unfolded a whole set of new possible solutions in the sensing and biosensing fields, as it offers the unprecedented possibility to obtain a wide range of low-cost, referenceless, and ultrasensitive devices using the same technological approach. The potentials in network electrophysiology and (neuro)pharmacology of such a versatile device have been preliminarily explored, and the proposed approach turned out to be suitable for both the detection of the electrical activity of living cells and the monitoring of small pH variations, the latter application being possible thanks to the super-Nernstian sensitivity of the sensor given by the peculiar double-gated structure of the device itself. Further exciting developments are foreseen by the combination of different sensing capability into the same platform, thus paving the way to the development of low-cost and easy-to-fabricate multisensing tools for cellular applications. However, the possibility of implementing disposable smart-petri dishes with a specific substrate functionalization and containing a multiparametric sensor array based on OCMFET is only one of the possible applications of this system. In fact, precoated and sterile MOA petri dishes can be foreseen as standard tools for neurophysiological studies as well as neuropharmacological and neurotoxicity assays. Moreover, the use of such devices in combination with stem-cell technology for developing new brain-on-a-chip methods for applications in precision medicine (i.e., patient specific studies-therapies) is an additional future application of this organic transistor-based technology.

On a more long-term perspective, thanks to the conformability flexibilities of the substrates on which these devices can be fabricated, possible applications can be foreseen in the field of in vivo brain interfaces and neuroprosthetics. In fact, as recently demonstrated (Viola et al. 2018) OCMFET devices can be easily fabricated onto sub-micrometer substrates, this feature not only allowing the device to conformably cover basically any surface but also conferring on it an incredible

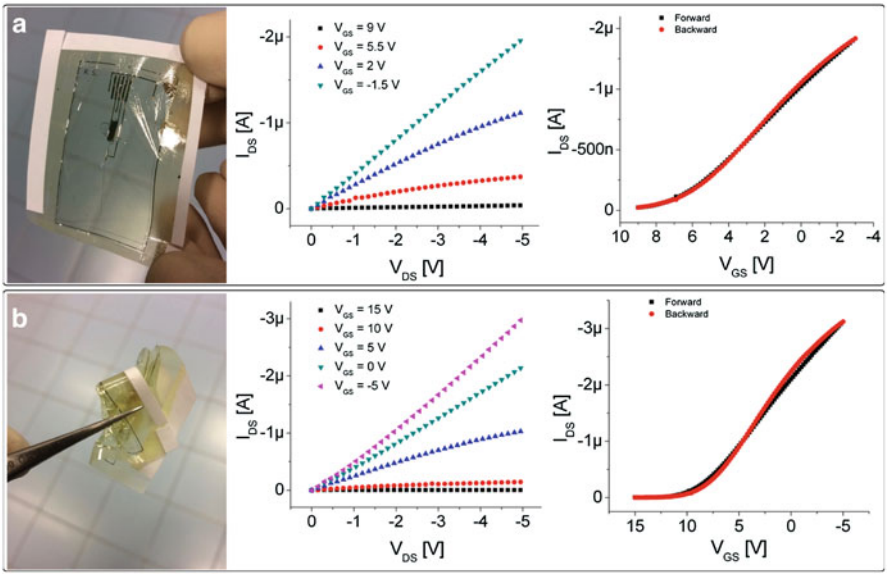

Fig. 5 Characterization of an ultraflexible OCMFET before (**a**) and after (**b**) the exertion of a mechanical stress. The device, thanks to the submicrometer substrate, showed an excellent retention of its electrical characteristics

resistance to mechanical stress (as presented in Fig. 5). In conclusion, although other organic transistor-based devices have been already used for in vivo measurements (Khodagholy et al. 2013, 2015), the OCMFET, thanks to its unique structure and its referenceless nature, represents a very promising and interesting alternative for both acute and chronic clinical neural applications.

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