Sensing Cellular Metabolic Activity via a Molecular-Controlled Semiconductor Resistor
Ilina Kolker Baravik, † Eyal Capua, † Elena Ainbinder, ‡ and Ron Naaman*†‡

†Department of Chemical and Biological Physics and ‡Department of Life Sciences Core Facilities, The Weizmann Institute of Science, Rehovot 76100, Israel

Supporting Information

ABSTRACT: Over the last decade, we have developed a molecular-controlled semiconductor resistor (MOCSER) device that is highly sensitive to variations in its surface potentials. This device was applied as a molecular sensor both in the gas phase and in solutions. The device is based on an AlGaAs/GaAs structure. In the current work, we developed an electronic biosensor for real-time, label-free monitoring of cellular metabolic activity by culturing HeLa cells directly on top of the device’s conductive channel. Several properties of GaAs make it attractive for developing biosensors, among others its high electron mobility and ability to control the device’s properties by proper epitaxial growing. However, GaAs is very reactive and sensitive to oxidation in aqueous solutions, and its arsenic residues are highly toxic. Nevertheless, we have managed to overcome this inherent chemical instability by developing a surface-protecting layer using polymerized (3-mercaptopropyl)-trimethoxysilane (MPTMS). To improve cell adhesion and biocompatibility, the MPTMS-coated devices were further modified with an additional layer of (3-aminopropyl)-trimethoxysilane (APTMS). HeLa cells were found to grow successfully on these devices, and MOCSER devices cultured with these cells were stable and sensitive to cellular metabolic activity. The sensitivity of the MOCSER device results from the sensing of extracellular acidification in the microenvironment of the cell-MOCSER interspace. We have found that this sensitivity is maintained only when the device is partially covered with the cellular layer, whereas at full coverage the sensitivity is lost. This phenomenon is related to the negatively charged cellular membrane potentials that lead to a reduction in the channel’s conductivity. We propose that the coated MOCSER device can be applied for real-time and continuous monitoring of cellular viability and activity.

INTRODUCTION

Hybrid organic–inorganic electronic biosensors1–3 are an attractive tool for monitoring chemical/molecular processes; thus, they could assist in a wide range of applications, such as developing and screening new drugs4,5 and detecting hazardous toxins.6 The importance of developing cell-based biosensors, despite the limitations imposed by the limited life span of single cells, is that such systems, based on living cells, can directly reveal physiological and functional information about the living state of cells and their response to external, physical, and chemical stimuli.7,8

The state of living cells can be monitored by several methods.9–11 One of the main methods is to monitor the cellular metabolism.12–14 Cells consume energy to maintain vital cellular functions, such as synthesizing various compounds and for maintaining their transcellular membrane gradients. This energy consumption leads to the creation of metabolic end products, such as CO2 and lactic acid. As a result, the extracellular medium (ECM) becomes acidified15–19. For example, under steady-state conditions, a single cell produces ~105 protons per s.20 Respiration is the major pathway for the production of ATP from glucose in vivo, whereas glycolysis predominates in vitro,19,21 with a greater extent of ECM acidification.

Silicon-based electrolyte oxide field-effect transistors (FETs), especially ion-selective FETs, are the most commonly used transducers for monitoring this acidification process.22–24 In FET devices, the presence of molecules defines the gate potential, which alters the channel’s conductivity. The shortcomings of FET-based devices are mainly low sensitivity, owing to drift at the gate electrode, and lack of stability in harsh environments.25

Two decades ago, we proposed the molecular-controlled semiconductor resistor (MOCSER) technology, a FET-like device, in which the gate electrode was replaced by a molecular layer adsorbed directly on the semiconductor surface.26–29 This device is based on a two-dimensional electron gas structure of AlGaAs/GaAs, and it was applied for developing molecular sensing systems both in the gas phase30,31 and in solutions.32 Several properties make this technology useful for developing...
molecular sensors, among them is the high electron mobility channel in the form of 2D electron gas and the ability to obtain enhanced sensitivity by proper band gap engineering and epitaxial growth. Moreover, by applying the relevant surface chemistry, it is possible to directly adsorb molecules on top of the GaAs semiconductor without an oxide intermediate, thus enabling sensing mechanisms directly through surface states. The electronic properties of the adsorbed molecules define the surface chemical potential, which leads to a change in the device’s surface states, which in turn defines the band bending in the semiconductor and affects the currents passing through it.32,35

One of the drawbacks of GaAs is its susceptibility to form an unstable oxide under ambient conditions. In an aqueous environment, this oxide formation may be even more prominent, resulting in the release of toxic arsenic species.37,38

However, we have managed to overcome the inherent chemical instability of GaAs by developing a surface-protecting layer using a polymerized 3-mercaptopropyl-trimethoxysilane (MPTMS) layer. This layer was polymerized on top of the device, thus avoiding the direct exposure of the GaAs surface to water or humidity.35,39

In the present work, we aimed to develop a cell-based biosensor, using the MOCSER technology, by culturing cells directly on top of the device’s active channel. This technology enables a real-time and label-free monitoring of the cellular viability by sensing cellular metabolic activity.

**EXPERIMENTAL SECTION**

Human HeLa, cervical adenocarcinoma cell line, was used to perform the experiments. The cells were cultured according to standard protocols used by the American Type Culture Collection (ATCC) organization. The culturing was carried out in Dulbecco’s modified Eagle’s medium (DMEM, Gibco cat. no. 41965). The culture medium for the cells was a CO2-independent medium (Gibco, cat. no. 18045-054), thus allowing us to conduct the electrical measurements without the need for a constant CO2 supply. Both media, namely, DMEM and CO2-independent medium, were supplied with 1% of 200 mM l-glutamine (Biological Industries, cat. no. 03-020-1B), 10% of fetal bovine serum (Gibco, cat. no. 12657-029), and 1% of penicillin/streptomycin antibiotics solution (Biological Industries). The cells were passaged every 2–3 days, using a trypsin–ethylenediaminetetraacetic acid (EDTA) solution (0.25% trypsin–0.02% EDTA, Biological Industries, cat. no. 03-050-1B), when 80% confluency was reached.

**Device Fabrication.** MOCSER devices were fabricated based on a preexaminied AlGaAs/InGaAs/GaAs pseudomorphic high electron mobility transistor (MOCMOS) structure.32,35 The evential devices were designed to carry 16 conductive channels, having dimensions of 600 μm long by 200 μm wide, separated by 200 μm uniform spaces, to minimize cross-talking and leakage of currents between the channels. The devices were fabricated by standard photolithography procedures. Ohmic contacts were made by the e-beam evaporation of metallic layers for forming n-type ohmic contacts as follows: Ni/Au/Ge/Ni/Au (50/600/300/200/1000 Å), respectively. The conductive channels were isolated by mesa etching with a soft piranha solution of H2O/H2O2/H2SO4 (40:8:1) to finally ensure a flow of current solely between the device contacts. Finally, the device was coated with a 30 nm passivation layer of Al2O3, and selective openings were made to expose the pads for wire bonding and the channels for molecular sensing. The resulting device produced a general resistance of about 2.5 kΩ, with a contact resistance of 1 ± 0.3 Ω-mm and a sheet resistance of 850 ± 45 Ω.

**Molecular Modification with APTMS–MPTMS.** The GaAs-based MOCSER devices were coated using a protective and functionalized layer of MPTMS according to a procedure that was previously developed in our lab.39 The resulting 15–18 nm layer allowed the MOCSER device to operate continuously and in a stable manner in harsh biological environments.33–35 The MPTMS polymer surface was further modified with an additional layer of APTMS, to improve cell adhesion and biocompatibility. Briefly, the MOCSER devices coated with MPTMS were treated with an ultraviolet ozone cleaning system for 20 s to hydroxylate the surface, followed by the addition of 50 μL/mL of APTMS in ethanol, with 75 μL/mL of NH4OH as a condensation agent. The samples were kept at room temperature for 24 h. The resulting additional APTMS polymer thickness was estimated by ellipsometry measurements to be 5 nm.

**Experimental Setup and Apparatus.** To encapsulate the MOCSER device and to conduct wet electrical measurements and cell cultivations, an assembly consisting of a Petri dish above a polydimethylsiloxane (PDMS) well (5 × 5 × 5 mm) was constructed and used (see Figure 1). This assembly was mounted on top of the MOCSER device, using a biocompatible UV-curable adhesive NOA81 (Norland Optical Adhesives).

**Figure 1.** (A) Schematic representation of the encapsulated MOCSER device, where cells are cultured within a PDMS well assembly. (B) Schematic representation of the experimental setup for real-time, electrical monitoring of cellular activity under well-controlled physiological conditions.

Before cell cultivation, the encapsulated MOCSER was disinfected with 70% ethanol. The device was then preincubated for 20 min with a growth medium solution of DMEM. Incubation was carried out in a humidified 37 °C incubator with 5% CO2. The cultured cells were then treated with a trypsin–EDTA solution and passaged to the MOCSER assembly. The passage was performed by pipetting 100 μL of the cell culture solution containing 5 × 105 cells; the cell number was determined using a TC20 automated cell counter (Bio-Rad #145-0101). The cells were cultured on the device for 16 h in a humidified 37 °C incubator with 5% CO2 in DMEM to allow the cells to adhere to the surface of the MOCSER device. After a 16 h preincubation period, the medium above the cells was exchanged with a CO2-independent medium. Finally, the whole system was transferred to a mini-incubator.
that maintained the experimental apparatus under a controlled environment of 37 °C, and the measurement was initiated.

**Electrical Measurements.** Sixteen channels per device were measured simultaneously using a Keithley 2636A source measure unit and a home-built switch control box, which was controlled and monitored by a LabVIEW program. The HeLa cellular metabolic activity was measured over 6 h using pulsed cycles at a constant source-drain voltage of 0.6 V. Within the pulses, the “on” state was set to 3 min (the time needed for the signal to stabilize), whereas the “off” state was set to 20 min to reduce the electrical load on the cells, yet enough to maintain measurable electrical currents and responses.

To test the device’s sensitivity in the CO₂-independent medium solution, calibration plots were measured for various pH solutions. All pH measurements throughout the experiments were performed using a commercial pH meter (portable pH-meter 1120 with a 3 mm InLab 423 combination pH micro electrode, Mettler Toledo International, Inc.). Moreover, source-drain current ($I_{sd}$) versus voltage ($V_{sd}$) characteristics at various gate voltages ($V_g$) were applied using an external gate. Here, to apply gate voltages without currents, a Pt wire electrode was coated with a Si$_3$N$_4$ dielectric layer of 300 nm, using a plasma-enhanced chemical vapor deposition tool. These measurements were conducted both on bare MOCSERs and on MOCSERs coated with a monolayer of HeLa cells.

**Microscopic Imaging.** Optical microscopy images of HeLa cells cultured on top of the MOCSER device were acquired using an Olympus BX-62 microscope in a bright-field/reflection mode, using a 10x air objective. The images were recorded with a complementary metal-oxide semiconductor (CMOS) camera (Neo sCMOS; Andor Technology) using Andor Solis software.

**Measurement of the Extracellular Acidification Rate (ECAR) by Using the Seahorse XF Analyzer.** The ECAR was measured by using the Seahorse XFe96 analyzer (Agilent Technologies). Briefly, HeLa cells were cultured in a dedicated 96-well plate at a seeding concentration of 1 × 10⁴ cells/well. The culturing was carried out in DMEM growth medium for 16 h in a humidified 37 °C incubator with 5% CO₂. Before measurements were taken, the growth medium was exchanged with a nonbuffered XF base medium (Seahorse Bioscience, cat. no. 102353-100) containing 4 mM l-glutamine. The ECAR was measured under basal conditions and in response to different glucose concentrations ranging from a 0.1 to 10 mM (2.5 M o- (+)-glucose solution, Sigma-Aldrich, G8769). The ECAR was reported as an absolute acidification rate (i.e., ΔpH/min) and normalized against cell counts using the Hoechst reagent (Molecular Probes, cat. no. H3750).

## RESULTS AND DISCUSSION

**Culturing of HeLa Cells on the MOCSER Device.** HeLa cells were successfully cultured on top of GaAs surfaces and MOCSER devices coated with MPTMS or APTMS–MPTMS polymers. Figure 2 shows optical microscopy images of HeLa cells grown on top of four different samples: uncoated GaAs substrate (i.e., negative control; Figure 2A), a standard culturing Petri dish (i.e., positive control; Figure 2B), a GaAs substrate coated with MPTMS (Figure 2C), and a GaAs substrate coated with APTMS–MPTMS (Figure 2D). Figure 2C shows that cells adhered suboptimally to the surfaces coated with the MPTMS layer. This is due to the partially hydrophobic nature of the MPTMS layer (having a contact angle of 70°). Surfaces coated with APTMS–MPTMS (Figure 2D) were more hydrophilic (i.e., having a contact angle of 45°), allowing better adherence of cells. This is due to the introduction of...
amine groups that enhance cell adhesion and thus provide favorable biocompatibility for the cellular layer. In addition, the cells grown on bare GaAs substrates exhibited an abnormal HeLa epithelial morphology, with a low density of cells. The cells grown on the surfaces coated with a layer of APTMS—MPTMS exhibited morphology and density similar to that exhibited in a standard Petri dish.

The above-mentioned polymers not only provide a chemical passivation layer against the etching of GaAs in aqueous solution, but also serve as an electrically stable receptor layer with different functionalized groups, such as negatively charged thiols and positively charged amines that are susceptible to pH variations.

**I–V Characterization of the MOCSER Device.** Figure 3 shows the obtained source–drain current ($I_{sd}$) versus the source–drain voltage ($V_{sd}$) at various gate voltages ($V_{gs}$) for a MOCSER device coated with a monolayer of HeLa cells (the continuous dotted line) compared with a bare MOCSER device (the dotted line). In this set of measurements, the $V_{gs}$ was applied using a platinum wire electrode just above the channel. The gate voltages were increased up to the point of gate current leakage of 250 nAmp. The electrical measurements were performed in a CO$_2$-independent medium solution at pH 7.2. These $I–V$ characteristics show that even in a physiological environment, the GaAs MOCSER device maintains its n-type depleted-mode characteristics. It can be clearly seen that at $V_{gs} = 0$ V, the current drops, for a device coated with a monolayer of HeLa cells, to a point that represents an almost closed channel. This drop is related to channel modulation attributed to the accumulation of negatively charged cell membranes on the channel surface. When $V_{gs} = 1$ V is applied, the channel’s resistance is restored to its original resistance. On the other hand, by applying negative voltages on the bare device, the current drops to the base current of the cell-coated devices (see Figure S1).

**pH Sensitivity and Stability under Physiological Conditions.** Because the immediate measure of cellular activity is related to the release of acidic byproducts by the cells, we conducted a calibration measurement in which a CO$_2$-independent medium (pH 7.2) was titrated with hydrochloric acid down to pH 1 (see Figure 4) with increments of 1 pH unit. Figure 4 shows that the current of the conductive channel, $I_{sd}$, increases with the acidity of the solution (decreasing the pH). This titration plot exhibits a sensitivity of about $-4 \mu$A/pH. The shoulder in the plot, at pH = 6, is related to the composition of the CO$_2$-independent medium, containing a unique buffering system composed of mono- and dibasic sodium phosphate (pK$_a$ 6.85 at 25 °C) and β-glycerophosphate (pK$_a$ 6.30 at 25 °C).

The MOCSER response exhibited a similar trend, when a standard phosphate buffer solution (150 mM) was titrated within its buffer zone with only a sensitivity of about $-7.5 \mu$A/pH (see Figure S2).

The MOCSER response shows a logarithmic dependence on the concentration of protons in the solution. This behavior can be explained by utilizing the site-binding model and based on the Nernst equation, in which the surface potential is affected by the ionic strength of the solution. The pH response of MOCSER was found to be similar to that of APTMS–MPTMS polymer-modified MOCSER devices. It has been shown that the resistance of the channel is determined by the change in the surface/electrolyte interfacial potential ($\Delta\psi$). These potential changes are caused by surface protonation; therefore, both $\Delta\psi$ and $\Delta I$ correspond to the $\Delta$(pI – pH) term, where the pH value is defined for a tested solution and the pI value is the isoelectric point of the polymer coating the surface. When the pH of a tested solution is above/below the pI value of the surface, the surface becomes either protonated or deprotonated, respectively. The pI value of APTMS was found to be 2.1, whereas the pI value of MPTMS is 9.7. Because the amine groups of the APTMS layer are expected to be protonated, over the studied pH range, the change in current should be mainly defined by the protonation/deprotonation of the MPTMS and the APTMS polymer layers excluding the amine groups.

**Demonstration of HeLa Metabolic Activity through ECM Acidification.** To confirm that the HeLa cellular metabolic activity leads to ECM acidification, the Seahorse XFe96 analyzer was used. Briefly, the ECARs were measured for various glucose concentrations of 0.1, 1, and 10 mM over a dense coverage of cells (see Figure S3). Because the ECAR assay actually measures the pH of the bulk medium, the assay medium should be free of sodium bicarbonate and should possess a low buffer capacity. Therefore, before performing an assay, the growth medium was exchanged with a nonbuffered
Figure 5 shows that the HeLa cell line used was metabolically active and that its metabolic activity increases with increasing glucose concentrations.

Sensing HeLa Cellular Metabolic Activity by the MOCsER Device. For real-time monitoring of the HeLa cellular metabolic activity, the MOCsER devices were cultured with HeLa cells on top of the MOCsER devices. The activity was tested by measuring the cellular-coated devices in a commercial CO$_2$-independent medium, supplemented with a glucose concentration of 0.7 g/L (4 mM). The devices were measured for 6 h by electrically pulsed cycles. In parallel with the electrical measurements, the pH of the buffer solution, above the cellular layer, was found to be 7.0 ± 0.2 throughout the experiments as probed by a commercial pH meter. This result is explained by the presence of a buffering system in the bulk medium.

Figure 6 shows the typical output signals as a function of time obtained from different MOCsER devices when their channels were covered with different concentrations of cells, ranging from 0 to 100%. It can be clearly seen that on partially covered MOCsER devices (red and blue dotted curves; Figure 6A and their corresponding images; Figure 6B), an increase in the source–drain current was measured in response to the cellular metabolic activity, whereas no response could be measured for a fully covered device (black curve). The most significant response was obtained with 25% cell coverage, in which the source–drain current, $I_{sd}$, increased by more than 30 μA.

We attribute this increase in current to the acidification process occurring in the cell-MOCsER interspace. We propose that the acidic byproducts, released by the cells, diffuse into the surface-coated polymer and protonate the charged species embedded in the APTMS–MPTMS polymer layer. Consequently, this layer becomes more positively charged, until the proton equilibrium in the interspace is reached. This process occurs in the uniquely created cell-MOCsER interspace regions (see Figure 7), where protons are secreted by the cells and are instantly sensed by the MOCsER device. Here, the buffering environment is not effective in neutralizing the acid. This mechanism is further supported by measurements obtained when using the Seahorse XFe96 analyzer, in which the sensor probe is located as close as 200 μm above the cells; however, it could not detect changes in ECAR with the elevated glucose concentration in the same CO$_2$-independent medium.

Figure 6 also indicates that an inverse relation exists between the cell density on the MOCsER’s channels and the base currents, as well as the device’s sensitivity to extracellular acidification. For example, with increasing cellular density, the device’s base current and the sensitivity to extracellular acidification decrease. The black dotted curve in Figure 6A and its corresponding image represent a fully covered MOCsER channel, where no significant changes in $I_{sd}$ occur. This result is consistent with the lowering of the MOCsER channel’s sensitivity owing to the negative potential of the cellular membranes that close the channel’s conductivity. The loss of sensitivity observed here is not a result of a lack of cellular viability because:

(a) The cellular morphology, as seen by a light microscope, is in accordance with the epithelial morphology of living cells (lower image of Figure 6B as compared to that of Figure 2).

(b) The measurements conducted by the Seahorse metabolic analysis method clearly indicate that the cells are still very active on a fully dense well (see Figure S3).

(c) After treatment with 0.1% Triton X-100, a lysing agent that leads to the detachment of cells from the device surface (see Figure S4), the base current is recovered.
This above-mentioned unique sensing mechanism of our device, namely, its ability to sense cellular metabolic activity in the cell-MOCSER interspace, makes it especially suitable for monitoring cellular viability, with the ability to detect changes in cellular viability instantaneously, as compared to methods that rely on the sensing of changes in the bulk medium.

The MOCSER device, with its present parameters, suits for monitoring few cells because its sensitivity decreases at high cellular concentrations on the channel. However, in principle, the parameters of the device can be modified to overcome this limitation.

In the present study, we developed a GaAs-based biosensor for measuring cellular activity and viability. Its inherent chemical instability was reinforced using a polymerized MPTMS protective layer, capped with APTMS for increasing its biocompatibility and for enabling cell culturing and cellular thriving on its surface. We presented a real-time and continuous monitoring of the metabolic activity of HeLa cells as a measure of cellular viability by sensing the extracellular acidification.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.7b01702.

MOCSER’s device characteristics; MOCSER’s sensitivity and stability; HeLa cell density for ECAR measurements using the Seahorse XF analyzer; and MOCSER device’s response to killing of HeLa cells with Triton X-100 (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: Ron.Naaman@weizmann.ac.il (R.N.).

ORCID

Ron Naaman: 0000-0003-1910-366X

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Professor David Bensimon for his fruitful ideas and discussions.

■ REFERENCES

(1) Bousse, L. Whole cell biosensors. Sens. Actuators, B 1996, 34, 270−275.
(2) Bhalla, N.; Jolly, P.; Formisano, N.; Estrela, P. Introduction to biosensors. Essays Biochem. 2016, 60, 1−8.
(3) Van Doren, B.; Mehta, J.; Bekarta, K.; Rouah-Martin, E.; De Coen, W.; Dubrueil, P.; Blust, R.; Robbens, J. Recent advances in recognition elements of food and environmental biosensors: A review. Biosens. Bioelectron. 2010, 26, 1178−1194.
(4) Bhalla, N.; Di Lorenzo, M.; Pula, G.; Estrela, P. Protein phosphorylation analysis based on proton release detection: Potential tools for drug discovery. Biosens. Bioelectron. 2014, 54, 109−114.
(5) Yeung, C.-K.; Ingebrandt, S.; Krause, M.; Offenhäuser, A.; Knoll, W. Validation of the use of field effect transistors for extracellular signal recording in pharmacological bioassays. J. Pharmaceut. Toxicol. Methods 2001, 45, 207−214.
(6) Gavrilescu, M.; Dennerová, K.; Aamand, J.; Agathos, S.; Fava, F. Emerging pollutants in the environment: present and future challenges in biomonitoring, ecological risks and bioremediation. Nat. Biotechnol. 2015, 32, 147−156.
(7) Casey, J. R.; Grinstein, S.; Orlowski, J. Sensors and regulators of intracellular pH. Nat. Rev. Mol. Cell Biol. 2010, 11, 50−61.
(8) Kraus, M.; Wolf, B. Physicochemical microenvironment as key regulator for tumor microevolution, invasion, and immune response: Targets for endocytotechnological approaches in cancer treatment. Endocyt. Cell Res. 1998, 12, 133−156.
(9) Wolf, B.; Brischwein, M.; Baumann, W.; Ehret, R.; Kraus, M. Monitoring of cellular signalling and metabolism with modular sensor-technique: the PhysioControl-Microsystem (PCM). Biosens. Bioelectron. 1998, 13, 501−509.
(10) Gopal, K. V. Neurotoxic effects of mercury on auditory cortex networks growing on microelectrode arrays: a preliminary analysis. Neurotoxicol. Teratol. 2003, 25, 69−76.
(11) Maher, M.; Pine, J.; Wright, J.; Tai, Y.-C. The neurochip: a new multielectrode device for stimulating and recording from cultured neurons. J. Neurosci. Methods 1999, 87, 45−56.
(12) Mohri, S.; Shimizu, J.; Goda, N.; Miyasaka, T.; Fujita, A.; Nakamura, M.; Kajiy, F. Measurements of CO2, lactic acid and sodium bicarbonate secreted by cultured cells using a flow-through type pH/CO2 sensor system based on ISFET. Sens. Actuators, B 2006, 115, 519−525.
(13) Mohri, S.; Yamada, A.; Goda, N.; Nakamura, M.; Naruse, K.; Kajiy, F. Application of a flow-through type pH/CO2 sensor system based on ISFET for evaluation of the glucose dependency of the metabolic pathways in cultured cells. Sens. Actuators, B 2008, 134, 447−450.
(14) Parce, J. W.; Owicki, J. C.; Kercso, K. M.; Sigal, G. B.; Wada, H. G.; Mair, V. C.; Bousse, L.; Ross, K. L.; Sikic, B. I.; McConnell, H. M. Detection of cell-affecting agents with a silicon biosensor. Science 1989, 246, 243.
(15) Kemp, G. Lactate accumulation, proton buffering, and pH change in ischemically exercising muscle. Am. J. Physiol.: Regul. Integr. Comp. Physiol. 2005, 289, R895.
(16) Hochachka, P. W.; Mommsen, T. P. Protons and anaerobiosis. Science 1983, 219, 1391−1397.
(17) Warburg, O. H.; Dickens, F. The Metabolism of Tumours; Constable: London, 1930.
(18) Wu, M.; Neilson, A.; Swift, A. L.; Moran, R.; Tamagjne, J.; Parslow, D.; Armistead, S.; Lemiere, K.; Orrell, J.; Teich, J.; Chomicz, S.; Ferrick, D. A. Multiparameter metabolic analysis reveals a close link between attenuated mitochondrial bioenergetic function and enhanced glycolysis dependency in human tumor cells. Am. J. Physiol.: Cell Physiol. 2007, 292, C125.
(19) Owicki, J. C.; Parce, J. W. Biosensors based on the energy metabolism of living cells: The physical chemistry and cell biology of extracellular acidification. Biosens. Bioelectron. 1992, 7, 255−272.
(20) McConnell, H.; Owicki, J.; Parce, J.; Miller, D.; Baxter, G.; Wada, H.; Pitchford, S. The cytosensor microphysiometer: biological applications of silicon technology. Science 1992, 257, 1906−1912.
(21) Warburg, O. On the origin of cancer cells. Science 1956, 123, 309−314.
(22) Lorenzelli, L.; Maresin, B.; Martinova, S.; Tedesco, M. T.; Vallé, M. Bioelectrochemical signal monitoring of in-vitro cultured cells by means of an automated microsystem based on solid state sensor-array. Biosens. Bioelectron. 2003, 18, 621−626.
(23) Baumann, W. H.; Lehmann, M.; Schwinde, A.; Ehret, R.; Brischwein, M.; Wolf, B. Microelectronic sensor system for microphysiological application on living cells. Sens. Actuators, B 1999, 55, 77−89.
(24) Lehmann, M.; Baumann, W.; Brischwein, M.; Gahle, H.-J.; Freund, I.; Ehret, R. Simultaneous measurement of cellular respiration and acidification with a single CMOS ISFET. Biosens. Bioelectron. 2001, 16, 195−203.
(25) Pourcie-Gouzy, M. L.; Sant, W.; Humenyuk, I.; Malaguin, L.; Dollat, X.; Temple-Boyer, P. Development of pH-ISFET sensors for the detection of bacterial activity. Sens. Actuators, B 2004, 103, 247−251.
(26) Kumar, V. K. Remedial and adaptive solutions of ISFET non-ideal behaviour. Sens. Rev. 2013, 33, 228−237.
(27) Gartsman, K.; Cahen, D.; Kadyshевич, A.; Libman, J.; Moav, T.; Naaman, R.; Shanzer, A.; Umansky, V.; Vilan, A. Molecular control of a GaAs transistor. *Chem. Phys. Lett.* 1998, 283, 301−306.
(28) Vilan, A.; Ussyshkin, R.; Gartsman, K.; Cahen, D.; Naaman, R.; Shanzer, A. Real-Time Electronic Monitoring of Adsorption Kinetics: Evidence for Two-Site Adsorption Mechanism of Dicarboxylic Acids on GaAs(100). *J. Phys. Chem. B* 1998, 102, 3307−3309.
(29) Thomas, E.; Rudich, Y.; Trakhtenberg, S.; Ussyshkin, R. Water adsorption by hydrophobic organic surfaces: Experimental evidence and implications to the atmospheric properties of organic aerosols. *J. Geophys. Res.: Atmos.* 1999, 104, 16053−16059.
(30) Vilan, M. R.; El-Beghdadi, J.; Debontridder, F.; Naaman, R.; Arbel, A.; Ferraria, A. M. D. Rego, A. M. B. Development of nitric oxide sensor for asthma attack prevention. *Mater. Sci. Eng., C* 2006, 26, 253−259.
(31) Capua, E.; Cao, R.; Suenik, C. N.; Naaman, R. Detection of triacetone triperoxide (TATP) with an array of sensors based on non-specific interactions. *Sens. Actuators, B* 2009, 140, 122−127.
(32) Capua, E.; Natan, A.; Kronik, L.; Naaman, R. The Molecularly Controlled Semiconductor Resistor: How does it work? *ACS Appl. Mater. Interfaces* 2009, 1, 2679−2683.
(33) Tatikonda, A. K.; Tkachev, M.; Naaman, R. Detection of triacetone triperoxide (TATP) with an array of sensors based on non-specific interactions. *Sens. Actuators, B* 2009, 140, 122−127.
(34) Capua, E.; Natan, A.; Kronik, L.; Naaman, R. The Molecularly Controlled Semiconductor Resistor: How does it work? *ACS Appl. Mater. Interfaces* 2009, 1, 2679−2683.
(35) Kumar, T. A.; Capua, E.; Tkachev, M.; Adler, S. N.; Naaman, R. Hybrid Organic-Inorganic Biosensor for Ammonia Operating under Harsh Physiological Conditions. *Adv. Funct. Mater.* 2014, 24, 5833−5840.
(36) Vilan, A.; Cahen, D. How organic molecules can control electronic devices. *Trends Biotechnol.* 2002, 20, 22−29.
(37) Sileo, L.; Martiradonna, L.; Brunetti, V.; Tasco, V.; De Vittorio, M. Gallium arsenide passivation method for the employment of High Electron Mobility Transistors in liquid environment. *Microelectron. Eng.* 2012, 97, 333−336.
(38) Kirchner, C.; George, M.; Stein, B.; Parak, W. J.; Gaub, H. E.; Seitz, M. Corrosion Protection and Long-Term Chemical Functionalization of Gallium Arsenide in an Aqueous Environment. *Adv. Funct. Mater.* 2002, 12, 266−276.
(39) Tkachev, M.; Anand-Kumar, T.; Bitler, A.; Guliamov, R.; Naaman, R. Enabling Long-Term Operation of GaAs-Based Sensors. *Engineering* 2013, 05, 1−12.
(40) Yates, D. E.; Levine, S.; Healy, T. W. Site-binding model of the electrical double layer at the oxide/water interface. *J. Chem. Soc., Faraday Trans.* 1 1974, 70, 1807−1818.