3D-integrated ultra-low volume passive microfluidics with Fully-Depleted Silicon-On-Insulator Ion Sensitive Field Effect Transistors for Multi-parameter Wearable Sweat Analyzers

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ABSTRACT

Wearable systems could offer noninvasive and real-time solutions for monitoring of biomarkers in human sweat as an alternative to blood testing. Recent studies have demonstrated that the concentration of certain biomarkers in sweat can be directly correlated to their concentrations in blood, making sweat a trusted biofluid candidate for non-invasive diagnostics. We introduce a fully on-chip integrated wearable sweat sensing system to track biochemical information at the surface of the skin in real time. This system heterogeneously integrates, on a single silicon chip, state-of-the-art ultra-thin body (UTB) fully-depleted silicon-on-insulator (FD-SOI) ISFET sensors with a biocompatible microfluidic interface, to deliver a “Lab-on-skinTM” sensing platform. A full process for the fabrication of this system is proposed in this work and demonstrated by standard semiconductor fabrication procedures. The system is capable of collecting small volumes of sweat from the skin of a human, and posteriorly passively driving the biofluid, by capillary action, to a set of functionalized ISFETs for analysis of pH level and Na⁺ and K⁺ concentrations. Drop-casted Ion Sensing Membranes (ISM) on the different sets of sensors on the same substrate enables multi-parameter analysis on the same chip, with small and controlled cross-sensitivities, while a miniaturized Quasi-Reference Electrode (QRE) sets a stable analyte potential, avoiding the use of a cumbersome external Reference Electrode (RE). The progresses on Lab-on-SkinTM technology reported here can lead to autonomous wearable systems enabling real-time continuous monitoring of the sweat composition, with applications ranging from medicine to lifestyle behavioral engineering and sports.

Recently, sweat has been attracting a lot of attention as a possible analyte to monitor the health status of an individual. Sweat is easily accessible, does not require invasive analysis and contains hundreds of trackable biomarkers, for some of which the correlation with their concentration on blood is fully demonstrated.¹⁻⁴

Many publications have reported the employ of wearable devices for sweat analysis: recently Gao *et al.* have described the fabrication of a wristband containing a set on functionalized electrodes on a flexible PCB, capable of measuring the concentration of glucose, lactate, sodium and potassium, as well as the skin temperature.⁵ Another wristband, though based on ISFETs and not including functionalization, has been proposed by Nagata and Takey,⁶ while other groups^{7,8} fabricated ion-sensing patches based on electrical or colorimetric detection.

All of these devices, however, exploit very large sensing areas, requiring sweat rates achievable only under intense activity conditions.^{9,10} Furthermore, no control over the sweat rate is provided, which prevents from retrieving reliable measurements in non-stable conditions. These issues have been tackled by some groups introducing a chemical-based stimulation for sweating, based on pilocarpine or carbachol.¹¹⁻¹³ This strategy, however, can influence the properties of the secreted sweat^{14,15} adding a further level of complexity to the system. We propose, as a more reliable and user-friendly alternative, the use of a microfluidic interface to enhance the collection of sweat, requiring lower volumes of liquid to fill the system. Our ISFET device relies on sensing areas with sizes in the order of few μm^2 and a microfluidic system which can be filled with less than 200 nl of sweat. The addition of a miniaturized QRE in the system allows having all the components needed for sensing on a chip smaller than 1cm^2 . The biomarkers chosen for this prototype, as stated, were sodium and potassium. The first is a useful marker to individuate hormonal changes which prelude ovulation,¹⁶ while the second can be used for diagnosing cystic fibrosis, as the ratio between the molar concentration of Cl^- and K^+ has recently been proposed as a more reliable diagnostic parameter compared to Cl^- concentration alone.¹⁷ Sodium concentration can be used to estimate the sweat rate¹⁸ and an increase of the ratio between potassium and sodium concentration in sweat might be a symptom of kidney failures.¹⁹

Fully depleted ultra-thin body silicon on insulator nanoribbon ISFETs for sensing applications

The development of metal oxide field-effect transistors (MOSFETs) eventually led to the study of the interface between oxides or metals and liquids which resulted in the development of the Ion Sensitive FET (ISFET). The ISFET was introduced in the 1970's²⁰ as an alternative to glass electrodes with aims to miniaturize sensing systems. The ISFET is similar to a planar metal oxide semiconductor field effect transistor (MOSFET) in many respects, the main difference being the gate electrode. The metallic gate of MOSFETs is absent in ISFETs, exposing directly the gate oxide to a liquid environment. The gate electrode is therefore replaced with a RE, and the potential at the silicon surface is a function of the RE bias and the influence of the ions or charged molecules inside a solution, as described by the following equation, in strong inversion.

$$V_G = E_{\text{Ref}} - \Psi_0 + \chi^{\text{sol}} - \frac{\phi_{\text{Si}}}{q} - \frac{Q_{\text{ox}} + Q_{\text{SS}} + Q_{\text{B}}}{C_{\text{ox}}} + 2\phi_F \quad (1)$$

Where V_G is the gate to source bias, E_{Ref} is the bias applied through the RE or QRE, Ψ_0 accounts for the ionic content of the analyte, χ^{sol} represents the surface dipole potential of the analyte, ϕ_{Si} refers to the silicon workfunction, q is the elementary charge, Q_{ox} , Q_{SS} and Q_{B} are, respectively, the oxide, interface and depletion layer charges, C_{ox} is the oxide capacitance and $2\phi_F$ is the surface potential at the silicon-oxide interface in strong inversion. The effective bias

applied on the gate of the ISFET is therefore linearly influenced by the ion concentration in the Liquid Under Test (LUT). In case of weak inversion operation, the surface potential of Field Effect Transistor (FET) varies between Φ_F and $2\Phi_F$ and the drain current depends exponentially on the gate to source bias. For instance, for pH sensing in weak inversion operation (at gate voltages smaller than the threshold voltage),²¹ the following equation stands:

$$I_D = I_{D0} \exp \left[\frac{q}{nkT} \left(V_G - \gamma - 2.3\alpha \frac{kT}{q} pH \right) \right] \quad (2)$$

Where I_D is the drain current of the ISFET, γ is a constant including the reference electrode potential, while the n-factor is the so-called slope factor of the ISFET including effects of the double layer and any other top passivation layer, if used. A functional operation of ISFETs in weak inversion operation offers ultra-low currents (below hundreds to tens of nA) and, therefore, low power consumption per sensor.^{22,23}

ISFET devices have become promising competitors with other sensing technologies as they allow integration with various interfacing electronic readouts being CMOS compatible. In addition, ISFETs provide the possibility of meeting the demand for multifunctional and scalable sensors with low fabrication costs. Furthermore, ISFET technology can be label-free, therefore there is no need to attach any label or probes to the analyte to improve the detection capabilities.²⁴ This characteristic provides the advantage of better real-time detection with high specificity and detection sensitivity if compared to other label-free sensors. In literature it is reported that this kind of sensors provides mechanical durability and resistance to the environment, making them reliable over time.²⁵

RESULTS AND DISCUSSION

In our microsystem, the n-type nanoribbon ISFETs are built on an Ultra-Thin Body Fully-Depleted Silicon-On-Insulator (UTB FD SOI) substrate, with a thin layer of Buried Oxide, (30 nm Si on 20 nm BOx), which allows good electrostatic control by eliminating the parasitic capacitances and reducing the OFF-state current by preventing the formation of parasitic current paths. The fabrication process is schematically represented in Fig. 1a) and will be explained in details in the method section. The final chip, shown in Fig. 1b), features sides of 9.1 mm.

Figure 1. a) Schematic fabrication process flow. b) Top view of a fabricated sensing chip with four sets of ISFETs. c) Zoom on the top view on the region with ISFETs, before microfluidics processing. d) SEM image of an UTBSOI ISFET. e) TEM image of a device section, taken at the edge of one of the dielectric openings created for electrical access, highlighting the gate stack. f) Miniaturized QRE. g) SEM image of the chlorinated silver surface.

Thirty-two ISFETs are placed in the chip and are divided in four groups that are independently functionalized for sensing different biomarker. A detail of one of these groups can be observed in Fig. 1c). A metal gate has been employed to evaluate the performances of the as-fabricated FETs: the transfer characteristics extracted showed the excellent I_{ON}/I_{OFF} ratio of 10^6 , with a nearly ideal SS of 65 mV/dec; the gate leakage remained within 10 pA (Fig. 2).

Figure 2. Transfer characteristics, I_D - V_G of a fabricated FET at $V_D = 100$ mV. The gate leakage, I_G , is negligible compared to the drain current over the full range of gate bias operation.

A layer of Silver is deposited on the chip, visible in Fig. 1f), to allow the integration of the QREs; the silver is then chlorinated to obtain an Ag/AgCl reference electrode, protected by a PVC membrane to extend its lifetime. The procedure to fabricate a miniaturized Ag/AgCl QRE is explained in the methods section of this paper.

Integration of ISFETs with a microfluidic interface for collection of low volumes of sweat

The integration of a microfluidic interface with the ISFET devices is paramount as it could enable the collection of sweat even at low-sweat-rate conditions. So far, wearable sweat sensors are tested on people performing physical activities,⁵⁻¹³ we propose the use of a patterned microfluidic interface to reduce the required volumes of liquid needed to perform sweat analysis, and thus trigger sweat analysis even when a person is at rest. Sweat rates in different parts of the body have been previously categorized:¹⁰ considering a human arm, the average sweat rate under exercise conditions is in the range of 1.5 microliters/cm²/minute, while, when at rest, the average sweat rate decreases to 20 nanoliters/cm²/minute. The microfluidic interface we propose in this work has a total volume capacity of 170 nanoliters, this is achieved by fabricating microchannels with widths of 50 micrometers, and a thickness of 25 micrometers. The integration of such a microfluidic interface aims for the collection of sweat even at low sweat rate conditions, which poses a significant advantage compared to what is reported in the literature.

A schematic of the fabrication process based on SU-8 photoresist, is shown in Fig. 3a). A first SU-8 passivation layer is deposited on top of the fabricated ISFETs to isolate the contact metal pads from the liquid flow and avoid any short-circuits. Afterwards, a second layer of SU-8 is deposited to pattern the passive microfluidic system. The SU-8 is a photosensitive resin with high mechanical resistance²⁶ and a measured contact angle (CA) of 64.1°, shown in Fig. 3b), low enough to passively drive the sweat by capillary forces.

As previously mentioned, this work aims to find a suitable interface that is compatible with human skin, therefore, biocompatibility of SU-8 becomes of the uttermost importance. Recent

studies have proven SU-8 as a material that offers biocompatible conditions such as already FDA approved materials like silicone elastomer, Buna-S and medical steel.²⁷

Figure 3. a) Schematics of the process flow to fabricate the passive microfluidic system and subsequent sensor functionalization and reference electrode coating. b) Measurement of the contact angle in SU-8 microfluidics.

The microfluidic system benefits from a complex design for sweat collection and transportation. The design includes an arborescent pattern with very thin channels enhancing capillary forces which drag the liquid droplets towards the sensors. Fig. 4 shows the design of the proposed microfluidic system. The sweat is gathered directly from the skin by an array of inlets, the biofluid travels then through the microfluidic channels, which bring it in contact with the QRE and the ISFETs, as shown in Fig. 4. The microfluidic channels end in a honey-comb structure such as the one shown on the top right of figure 4, which acts as a passive pump and a reservoir of liquid, promoting a continuous flow of sweat over a long period. The micropumps consist of an array of micropillars. The pillars change the capillary pressure inside of the system and thus allow controlling the speed of the liquid inside of the microfluidics. The pillars have a diameter of 80 μm , with 40 μm spacing between each other. Pillars are patterned as hexagons at regular intervals to achieve low flow resistance.

Figure 4. Lab On Skin™ concept. Center: 3D model of the presented system. Left: zoom in constituent of the microfluidic system on top of the UTB SOI FET sensors. Right: zoom in constituent inlets and outlets of a 30 μm thick layer of SU-8 in a 4" float wafer. The micropillars in the outlets create the largest capillary pressure in the microfluidic system and thus determine the flow rate in the system.

Figure 5 shows how the liquid is successfully collected by the arborescent structure and then transported through capillary forces to the outlet area. Experiments show that it takes only 1.74 seconds for the liquid to arrive to the drain material in the outlet of the microfluidics. An absorbent material is connected to the micropump to act as a waste reservoir. The absorbent material consists on a 60 μm thick layer of silk, this layer has high absorption capacity. Evaporation impedes to overcome the maximum capacity of the material, given the low volume of sweat channeled. To test the microfluidic devices, an artificial skin characterization setup was used as previously reported by the authors.²⁸

It is important to highlight the action of the micropump, as it diminished the velocity of the liquid front importantly compared to the microchannel. Figure 5 shows how the liquid takes less than 0.1 second to fill all the microchannels but it took approximately one more second to advance some micrometers inside of the micropump. These results led us to the conclusion that it is possible to collect low volumes of sweat on SU-8 microfluidics and to control the flow rate of the liquid inside of the system by using a patterned micropump.

Figure 5 Fluorescence micrograph of the passive collection and transport of a droplet of rhodamine dye solution through SU-8 microfluidics. The microbranches at the inlets increase the collection surface. Likewise, the sweat is transported through the capillary channels and then absorbed at a controlled rate by the passive microfluidic pump. An artificial skin characterization setup has been employed to characterize these devices.²⁸ Once full, the outlet discharges in an absorbent silk layer to enable continuous operation.

Functionalization of ISFET devices for sodium and potassium sensing

The fabricated UTB FD SOI FET sensors exploit a gate stack with a high-k dielectric (hafnium dioxide), corresponding to gate stacks used in today in advanced CMOS manufacturing.²⁹⁻³¹ HfO₂ has been chosen in the CMOS industry because of its large band offset with respect to silicon and high kinetic and thermodynamic stability.³² Hafnium dioxide, moreover, offers advantages in terms of pH sensitivity compared to other oxides, like the commonly used silicon dioxide, which reports less than ideal, non-linear threshold voltage shifts per pH.³³⁻³⁵ Thanks to the high density of binding states, HfO₂ offers a response to pH that is close to the Nernst limit (59 mV/pH at room temperature conditions).³⁶⁻³⁹ While the group of ISFETs dedicated to pH sensing have the HfO₂ dielectric layer of the gate directly exposed, the functionalized sensors' gate have a gold metal layer on top of which an Ion Sensitive Membrane is drop-casted. The ISMs are an effective functionalization method for chemical sensing in which a molecule is embedded in a PVC membrane which is then placed on top of the gate stack of the sensors. The molecule inside of the membrane chemically reacts to a specific ion and thus creates a barrier that prevents other ions from reaching the surface of the sensor (Fig.6). Only the specific ion for which the membrane has been functionalized can get through and give its charge to the ISFET's gate.⁴⁰ In the case of the potassium ISM, a PVC membrane is mixed with valinomycin, whereas for sodium ISMs, the PVC is mixed with Na ionophore X. A detailed process explaining how to obtain an ion sensitive membrane for sodium and potassium is provided in the methods section of this publication. The achieved thickness of the drop casted membranes was close to 8 μm .

Figure 6. Functionalization chemistry of Au gates with a polymeric membrane for potassium sensing. The ionophore is characterized by a size-based selectivity for the potassium ion, which is able to enter its molecular structure and then transported on top of the sensing surface, while non-specific ions are shielded out by the polymeric membrane.

Characterization of sensors with an external reference electrode

The nanoribbon ISFET devices have been tested with different pH buffers and sodium and potassium buffers with concentrations varying from 5 mM to 50 mM. The devices were first characterized with a commercial flow-through reference electrode and a PDMS stamp to test the performances of our fabricated sensors and ISM in controlled environment conditions. A detailed description of the characterization setup is described in the methods sections of this paper.

The pH sensing is performed with ISFETS having the HfO_2 top layer in contact with the LUT. The sensitivity to pH of our sensors has been evaluated measuring their transfer characteristics at different pH values and extracting the different RE biases (V_{REF}) at constant drain current ($I_D = 100 \text{ nA}$, for the results reported in this study). The drain bias V_D is kept constant at 200 mV for all the experiments. Operating the devices in the weak inversion regime of the FET has allowed us to work with sensors consuming as little as 20 nW of power, a characteristic highly desirable for wearable systems. The experimental results are reported in Fig. 7.

Figure 7. a) I_D - V_{REF} for different pH values. $V_D = 200$ mV. b) V_{REF} extracted at 100 nA for the five different pH buffers used in this characterization.

It can be noted that the measured sensitivity of our HfO_2 layer is relevantly lower than what has been reported by other studies^{41,42} or even by our previous publication.⁴³ We attributed this issue to the unavoidable material variabilities encountered in an university cleanroom. All the following measurements presented in this paper, however, are not based on HfO_2 , as the sensing is achieved with the functionalized gold interface, and thus do not suffer from this effect.

The sensitivities of the sodium and potassium-functionalized sensors to the concentration of their respective ions have been evaluated with an analogue method, employing a set of *ad hoc* prepared dilutions of NaCl and potassium KCl in deionized water. The transfer characteristics of the ISFETs are then extracted with each buffer solution. The results are presented in Fig. 8.

Figure 8. a) $I_D V_{REF}$ for different $[Na^+]$ values. b) $I_D V_{REF}$ for different $[K^+]$ values. $V_D = 200$ mV. The left-shift of the curves for higher concentration of specific salts is due to binding of positive charges on the sensing surface. A transfer characteristics with a non-specific ion is also plotted to show the efficiency of the functionalization.

The hysteresis of these curves (not represented here) has been evaluated measuring the distance, on the RE bias axis, between the transfer characteristics obtained sweeping V_{REF} from negative to positive values and vice versa. The values measured varied from curve to curve and ranged between less than 1 mV and 10 mV. This distance is due to the charges trapped in the membranes and on the sensing surfaces because of the wide bias sweep performed to extract the transfer characteristics. In real applications, when the full variation range of the gate bias will be maintained within less than 100 mV (corresponding to more than one order of magnitude variation in the ion concentration), this effect will be negligible. It can be observed that, for both sensors, a negative shift of the $I_D V_{REF}$ curves can be induced increasing the concentration of the specific salt, as it is expected for binding of positive charges on the sensing gates. In order to show the selectivity of the functional membranes for their specific ion, an $I_D V_{REF}$ curve is extracted employing an analyte with high concentration of non-specific ions; for both sensors, it can be observed that the negative shift of these curves is negligible, even when compared with the minimal specific salt concentration studied. The V_{REF} values corresponding to an I_D level of 100 nA have been extracted from the graphs and plotted in Fig. 9.

Figure 9. a) V_{REF} extracted at 100 nA for $[Na^+]$ b) V_{REF} extracted at 100 nA for $[K^+]$. Both graphs show a strong, logarithmic dependence of the threshold voltage value on the molar concentration of the specific ions. The sensitivities are extremely close to the Nernst limit.

Both sodium and potassium sensors show a strong, monotonic response to increases of their specific ions concentration. Excellent sensitivities have been obtained with a linear fit of the data, both reaching nearly-Nernstian values of, respectively, -62 and -55 mV/dec. Using these two graphs, it is possible to extract the concentration of specific ions which would correspond to the V_{REF} values extracted for the non-specific ions tested. The V_{REF} bias at which we get a current of 100 nA with 50 mM of KCl in the sodium-functionalized sensor is 0.276 V, corresponding to a NaCl concentration of 1 mM. For the 50 mM concentration of NaCl on the potassium-functionalized sensor we extracted a V_{REF} of -0.140 V, corresponding to a concentration of 0.29 mM of KCl. Defining the selectivity of our sensors as the ratio between the concentration of non-specific and specific ions needed to obtain the same V_{REF} value, we extract a selectivity of 50 for the sodium-functionalized sensor and of 172 for the potassium-functionalized sensor.

Monitoring the composition of the user's sweat requires the capability of performing real-time measurements. The most straightforward method to achieve this consists in fixing the potential of drain and RE to a known value (-100 mV in our case) and measuring the shifts in the drain current given by variations in ions concentration. The sodium and potassium measurements obtained with this strategy are shown, respectively, in figure 10 a) and b).

Figure 10. a) Real-time measurement of $[\text{Na}^+]$. b) Real-time measurement of $[\text{K}^+]$. Steep variations of the drain current can be observed upon switching to the salt dilution which is flown on the sensing gates. The current levels for a given salt concentration are constant, regardless which dilution had been used previously.

Ion concentration measurement with integrated quasi-reference electrode

Once the sensors were characterized with an external reference electrode, their performance was evaluated with the miniaturized reference electrode embedded in the chip. This test is used to observe if there is any significant degradation in the response of the ISFETs in a miniaturized system environment. An ISFET with an ISM membrane for potassium was characterized. The transfer characteristic of the devices are extracted at different KCl buffer concentrations to extract the sensitivity of the device to concentrations of K^+ (Fig.1 inset f). The results and the evaluated sensitivity, extracted at 100 nA, are shown in Fig. 11.

Figure 11. a) I_D - V_{REF} for different $[K^+]$ values. Inset shows a zoom in constituent demonstrating the low hysteresis in the response of the sensor (≈ 1 mV). b) V_{REF} extracted at 100 nA for $[K^+]$, showing ideal sensitivity and perfect linearity.

The transfer characteristics show a very low hysteresis (1-10 mV) in the response of the sensor and excellent linearity in terms of sensitivity, with a coefficient of determination close to 100% ($R^2 = 0,9995$) and fully-Nernstian response (-61 mV/dec) to changes in the potassium concentration, demonstrating the efficacy of our miniaturized QRE and the possibility of acquiring clean data from our fully integrated system. A real-time measurement employing the QRE has also been performed, with 0 V bias applied on the QRE, obtaining the graph shown in Fig. 12. We can highlight that, even with the miniaturized reference electrode, we were able to operate the sensors with low power consumption, as 200 mV were used to bias them.

Figure 12. Real-time measurement of $[K^+]$ with the embedded QRE. Even with a miniaturized QRE, steep variations of the drain current can be observed upon switching from one salt dilution to another, and the current levels for a given salt concentration are still independent from the previously measured dilutions.

It can be observed that, thanks to the low hysteresis of the devices (observed in fig 11a), equal levels of current are obtained for equal molar concentrations of salts, independently from the values of the previous measurements. The response time, up to 30 s, is clearly not due to the electronic properties of the sensor. We attributed it to the gradient-driven salt diffusion taking place inside the microfluidic channels and assumed it to be, in any case, absolutely compatible with the physiological variation rate of ion concentration in sweat. One might notice that the current levels are not coherent with the transfer characteristics shown in Fig.7a): this is because the ISMs have been removed and drop-casted again between the two measurements.

Integration with wearable systems for sweat sensing

The presented system can be potentially included in a wearable device, as it provides a full electrolyte sensing system with small form factor (0.79 cm^2) and a biocompatible interface to human skin, able to passively collect and manipulate low volumes of sweat ($\sim 20 \text{ nL}$). The use of a patterned microfluidic interface to human skin aims to trigger sweat analysis even when a person is at rest, we are reporting a CMOS compatible microfluidic system that takes this parameter into consideration for the system. Furthermore, the use of nanoribbon ISFETs enables to operate the sensors with very low power consumption (20 nW per sensor). A successful functionalization of the ISFET devices for sodium and potassium sensing has been shown, with close-to-Nernstian response sensitivities. The integration of all these functions delivers a low power consumption platform that could trigger multi-analyte sensing in real time with ultra-low volumes of sweat.

Table 1 summarizes the performances and capabilities of the introduced system in comparison with the state-of-the-art devices. All data presented in this table are reported as best achieved values. Although we do not report the most sensitive devices, we do report the use of low power consumption sensors for wearable sensing systems, which is critical to allow extended periods of autonomous operation of wearable systems. Table 1 reveals that in most of wearable sweat sensing systems, power consumption per sensor is not being reported. A key limitation of modern wearable systems is their dependency on batteries as power supply. Using low power consumption sensors can lead to longer autonomous operation times. Most of the sweat sensing systems presented in literature use Ion Sensitive Electrodes: while it is not established that ISFETs provide overall better performances in terms of sensitivity, they do provide a clear advantage in terms of power consumption.²⁰

Table 1 highlights another of the key advantages of the presented system. In literature, sweat sensing devices require subjects to be performing physical activity in order to produce large quantities of sweat. On-chip microfluidics such as the one presented in this work, could allow longer operation periods of the sweat sensing system and possibly enable sweat collection even when a person is at rest. This characteristic could allow non-interrupted sweat sensing analysis. Future works will include the use of other ion sensitive membranes to functionalize the nanoribbon ISFETs for detection of other electrolytes such as ammonia or calcium, as there are ionophores available in the market for this two electrolytes; other state-of-the-art wearable sweat sensors achieve sensing of other biomarkers such as glucose, lactate, chloride and alcohol as can be observed in Table 1.

TABLE 1. State-of-the-art of wearable sweat sensors

Reference number	K ⁺ sensitivity	Na ⁺ sensitivity	pH sensitivity	Detection of other biomarkers	Power consumption per sensor	μfluidic interface	Low sweat rate (~20nl/cm ² /min)
5	61.3 mV/dec	64.2 mV/dec	No	Glucose Lactate Temp.	Not reported	No	No
6	No	No	51.2 mV/dec	Temp.	~900 nW (calculated)	No	No
7	No	57 mV/dec	No	No	Not reported	Yes	No
8	No	No	N.A. colorimetric	Lactate Chloride Glucose	Not reported	Yes	No
11	No	No	No	Alcohol	Not reported	No	No
12	No	No	No	Chloride	Not reported	No	No
13	No	~49 mV/dec	No	Chloride	Not reported	No	No
This work	55 mV/dec	62 mV/dec	36 mV/dec	No	~20 nW	Yes	Yes

CONCLUSIONS

We presented a fully integrated device, embedding State-of-Art ISFETs, ISMs, miniaturized QRE and a low-volume passive microfluidics, for sweat-sensing applications. Device characterizations performed with an external flow-through RE have shown linear sensitivities of each group of sensors for their specific parameter of interest (pH, [Na⁺] and [K⁺]) and nearly-Nernstian sensitivity has been observed for the concentration of both sodium and potassium, demonstrating the high sensitivity of the functionalized membranes employed. Real-time measurements have also been performed, showing stable and repeatable readings with response times fully compatible with the physiological variation rate of ion concentration in sweat. The characterization of the potassium-functionalized sensors has been repeated employing the QRE embedded in the chip, resulting in excellent and astoundingly linear sensitivity. Real-time measurements of potassium concentration have been performed in a time interval of twenty minutes, showing reasonable stability and independence on previous molar concentration values. These results show the high potential of the heterogeneous device integration we presented, marking a further step forward towards commercial sweat-based wearable diagnostic systems.

METHODS

The ISFETs have been fabricated on a UTBSOI with a BOx thickness of just 20 nm. The fabrication process is depicted in Fig. 1a). The initial thickness of the silicon layer was 70 nm, thinned down to 30 nm with an oxidation-etching cycle, in order to improve the electrostatic control. Phosphorus implantation has been employed to define the S/D areas (5 keV energy, $7 \times 10^{14} \text{ cm}^{-2}$ dose), achieving a doping level of $2.2 \times 10^{19} \text{ cm}^{-3}$, evaluated from resistance measurements in *ad hoc* prepared structures. The physical shape of the FETs has been defined *via* photolithography and an extremely selective HBr-based dry etching, in order to accurately stop on the ultra-thin BOx.

A short dry oxidation step is performed before deposition, so that a thin pedestal oxide (5 nm according to the TEM images) is grown: this allows mitigating the charge-trapping issue at the interface between the ALD HfO₂ gate dielectric and the Si channels.⁴⁴ The gold gates and the silver reference electrodes are deposited by lift-off.

Ultra-low IBE is employed to open holes in the dielectric layer above the S/D areas, after carefully evaluating the etch rate for each material involved, then AlSi 1% is deposited by lift-off to create the S/D contacts.

The process performed to enable sensing on these devices is depicted in Fig. 3a.

First of all, the SOI surface is passivated with a 3 μm layer of SU-8, employing photolithography to leave contact pads, sensing gates and reference electrodes accessible. The microfluidic channels are built on top of the passivation layer with a second SU-8 layer and photolithography. The QRE is then chlorinated in a FeCl₃ solution and covered by a protective membrane, as reported by Emaminejad *et al.*⁴⁵ The sodium-selective membrane was prepared by mixing Na ionophore X (1% weight, w/w), Na-TFPB (0.55% w/w), PVC (33% w/w), and DOS (65.45% w/w). 100 mg of the membrane cocktail was dissolved in 660 μl of tetrahydrofuran. The potassium-selective membrane cocktail was composed of valinomycin (2% w/w), NaTPB (0.5%), PVC (32.7% w/w), and DOS (64.7% w/w). 100 mg of the membrane cocktail was dissolved in 350 μl of cyclohexanone.⁴⁶ Ion-selective membranes were then prepared by drop-casting 10 μl of the Na⁺-selective membrane cocktail and 4 μl of the K⁺-selective membrane cocktail onto the respective groups of ISFETs.

This setup is meant to exclude any non-ideality which is not coming from our sensors or sensing membranes, so that we can spot any issue with the fabricated device. Sensitivity measurements are carried out with pH buffers or *ad hoc* fabricated molar dilution of either NaCl or KCl. The analyte is inserted in the tubes *via* an 11 Elite Harvard apparatus syringe pump. Between two measurements, the channels are emptied but not cleaned, in order to avoid any favorable effect which wouldn't be present in real conditions.

The electrical measurements are performed with a cascade manual probe station (Cascade Microtech Inc., Summit 1200) and a precision semiconductor analyzer Agilent 4156A.

For real-time measurements, when a continuous flow is needed, an eight positions manual valve selector (C25-3188) is employed, allowing switching from one analyte to the other without generating bubbles in the tubes.

AUTHOR INFORMATION

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. ‡These authors contributed equally.

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