An On-Chip System to Monitor the pH of Cell Culture Media

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***Abstract*— We presents an ion sensitive field effect transistor to measure the pH of the cell culture media of human mammary adenocarcinoma (SKBR3). We use a drift mitigation technique that cycles the transistor to reset the drift in the system. We use to technique in the system to demonstrate an integrated system to monitor the pH continuously. As a part of the system a pulse width modulation circuit is designed in a 0.5 μm CMOS process which cycles the vertical electric field of the ion sensitive field effect transistor to reset the threshold voltage drift. We demonstrate the viability of a complete integrated system implementing our drift mitigation technique to monitor cultured cells. The integration is important in this application to allow for autonomous operation inside an incubator during cell culture.**

I. INTRODUCTION

The ion sensitive field effect transistor (ISFET) has revolutionized pH sensing since their discovery in 1970 by Bergveld [1]. Their size and lower power consumption make them good candidates for portable applications. Because they are suitable for fabrication in conventional CMOS processes, they have been able to take the advantage of the signal processing capabilities offered by CMOS. Due to these advantages they have been used extensively as a biosensor to measure and analyze biological signals [2] [3] [4].

Their commercial use has been limited due to the fact that they have an intrinsic threshold voltage drift [5] and challenges in packaging [6]. There has been substantial research to investigate the cause of the drift [7] [8] [9], but some of the factors causing the drift have not been fully understood. The drift has been shown to have temperature dependance [7], pH dependance [10] and sensing insulator dependance [9]. It was shown that one of the causes might be the accumulation of ions (H^+) at the silicon dioxide (SiO_2)-electrolyte interface [11].

This threshold voltage drift causes error in the measurement of the pH. ISFETs have to be calibrated over a period of time to compensate for the drift in the threshold voltage. Several methods have been used to compensate for the drift [12] [13] [14]. Our group previously reported a new method to mitigate drift. We found that cycling the vertical electric field could reset the drift [11]. Previously we have demonstrated this technique using software, but using this system inside an incubator for monitoring pH of a cell culture requires an integrated system. Here we demonstrate work towards building such an integrated platform to monitor the pH of the cell culture media precisely and continuously. For this a pulse width modulation circuit was designed in a 0.5μm CMOS process to be used in conjunction with the pH sensors to cycle the vertical electric field of the ISFET to reset the drift. The characteristics of the ISFET used in this work are shown in the figure 1. The ISFET has a sensitivity of 30 mV/pH as shown in the figure 1a.

The work also demonstrates the use of an ISFET to monitor the pH of the cell culture media. Molecular processes involved in cell cycle progression, cell proliferation, and differentiation are affected by the environmental acidity of the living cells [15]. Due to the increase in the metabolic activity of the tumor cells the microenvironment of the cells is intrinsically acidic [16] [17]. Thus monitoring the pH of tumor cells could potentially be used to for developing targeted medicine and Point-of-Care (POC) devices [18].

II. CELL CULTURE

The Human mammary adenocarcinoma (breast cancer) cell line (SKBR3), was used for our experiments. The growth media was made from ATCC-formulated McCoy's 5a medium with 10% fetal bovine serum. The cells were cultured in a standard incubator at 37° with 100% relative humidity.

The breast cancer cells were cultured on top of the ISFET shown in the figure 2c and 2d . This enables us to monitor the pH of the cell culture media. The output of the ISFET was measured and plotted as shown in the figure 2. Figure 2a shows decrease in pH of the cell culture media with the increase in the number of the cells. The change in pH is due to the increase in metabolic activity of the cells and production of lactic acid in the microenvironment of the cell [18].

III. DRIFT AND PULSE WIDTH MODULATION CIRCUIT

As discussed earlier ISFETs suffer from threshold voltage shift. The ISFET used for measuring the cell culture demonstrates a drift which is shown in the figure 1c. Cell growth requires precise control over the pH [6], and threshold voltage shifts might cause errors in the measurements. To mitigate this drift, it was shown that cycling the vertical electric field resets the threshold voltage drift [11]. Vertical electric field is controlled by the potential of the reference electrode and the substrate potential.

In [11] MATLAB® is used to control the reference and the substrate potential. Thus to cycle the vertical electric field on chip, a Pulse Width Modulation (PWM) circuit was designed in a 0.5 μm process as shown in the figure 3. A block diagram of PWM is shown in 3a and is composed of a voltage

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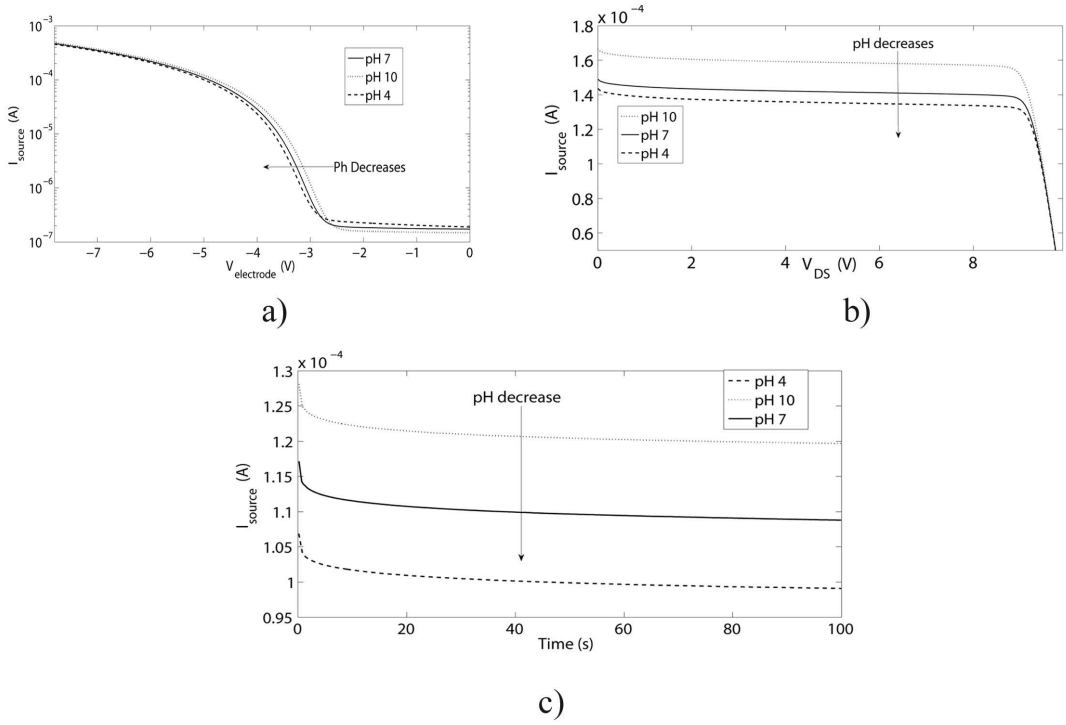

Fig. 1. a) Shows the transfer characteristics of the ISFET fabricated in SenSoNor process. The plot was obtained by varying the reference electrode potential and measuring the source to drain current of the ISFET. To obtain the sensitivity of the ISFET it was characterized with pH buffers having different value. A sensitivity of 30 mV/pH was obtained. b) Plot shows the source current with varying drain to source voltage and a constant reference potential. Varying the pH changes the surface potential and hence the change in saturation current. c) ISFETs suffer from threshold voltage shift which causes the drift in its channel current.

controlled oscillator, a 6 bit digital to analog converter, a 6 bit counter and a comparator. The layout for the PWM, shown in the figure 3b, occupies an area of 320 μm $\times$ 1000 μm. The layout area is huge because of the necessary size of the passive components in the voltage controlled oscillator, which are used to reduce the output frequency to mHz. The PWM circuit allows us to control the duty cycle of the output waveform as shown in the figure 3c and 3d.

Having an on chip voltage controlled oscillator allows us to control the frequency of the output by tuning the power supply as shown in the table I. Such a circuit allows us to reset the drift in the threshold voltage as can be seen in the figure 4. In the figure 4 the waveform in blue is where the vertical electric field of the ISFET, whose characteristics are shown in the figure 1, is cycled. The red curve in the figure 4 is for the ISFET operated continuously and hence has a drift in the threshold voltage.

Operating the pH sensors continuously results in a higher power consumption. Thus using the PWM circuit with a smaller on time and smaller frequency, which requires smaller power supply voltage according to table I, would result in a lower power consumption. Operating the PWM circuit at 2.25 volts consumes 74.7 μW of power, but the power consumption and the frequency increases with the power supply voltage. Since pH is a slow varying process, in cell culture media pH changes are over hours, it is sufficient to operate the PWM with a lower supply voltage, to perform measurements after 142 seconds in case of power supply of 2.25 volts.

TABLE I
POWER SUPPLY VS FREQUENCY

Power Supply (Volts)	Frequency (mHz)
5	400
4.5	150
4	77
3.5	62.5
3	23.51
2.75	11
2.5	8.6
2.25	7

IV. DISCUSSION AND FUTURE WORK

The work shows monitoring of pH in the cell culture media containing human mammary carcinoma cells, specifically SKBR3. A pulse width modulation circuit is used to cycle the vertical electric field of the ISFET by controlling the source, drain and the reference electrode potential of the ISFET. The die designed is shown in the figure 5.

In order to perform measurements in our previous work, the cells grown on top of the ISFET had to be taken out of the incubator which changes the temperature and the

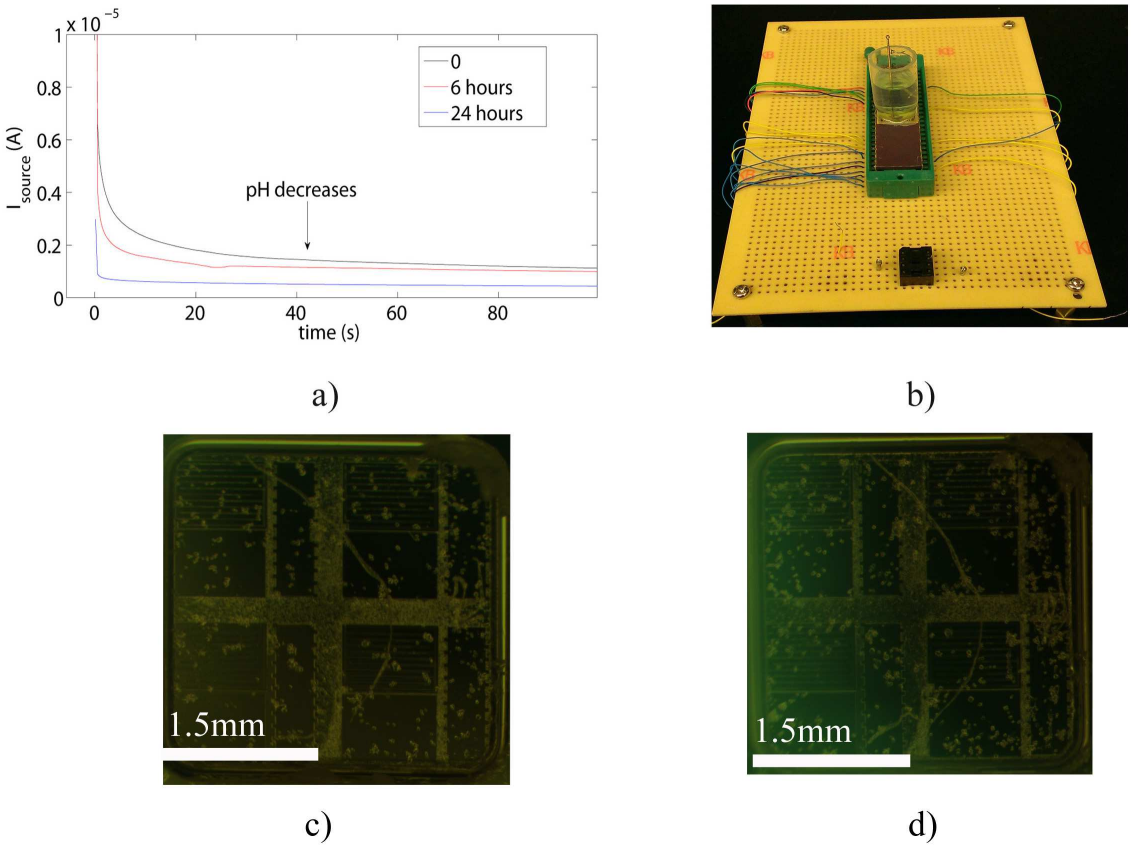

Fig. 2. a) The plot shows the decrease in pH, increase in the acidity of the microenvironment, with the growth of the human mammary adenocarcinoma (SKBR3) cells. b) The figure shows setup used to perform the measurements. c) The figure shows human mammary adenocarcinoma cells (SKBR3) growing on top of the ion sensitive field effect transistors. d) Cells after 24 hours of incubation on top of the ISFETs.

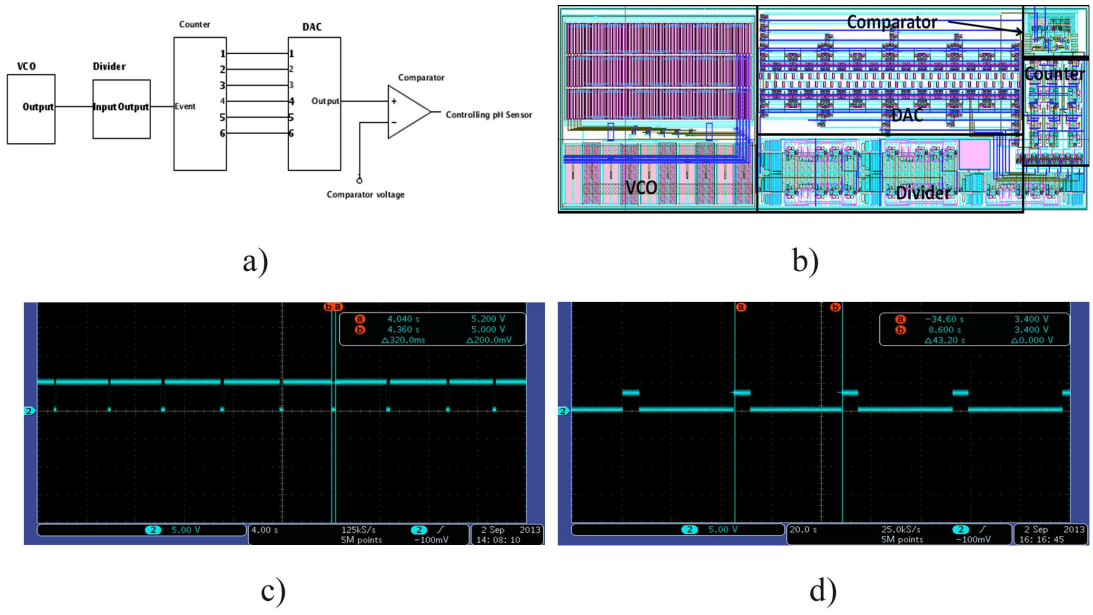

Fig. 3. a) Block diagram of the pulse width modulation circuit is shown with the individual components. b) The layout of pulse width modulation designed in a 0.5 μm process. The area of the layout is 320 μm $\times$ 1000 μm. c) Measured output of the pulse width modulation circuit with a higher on-time for continuous monitoring of the pH d) Measured output of the pulse width modulation circuit for larger off-time for lower power consumption and hence appropriate for portable applications

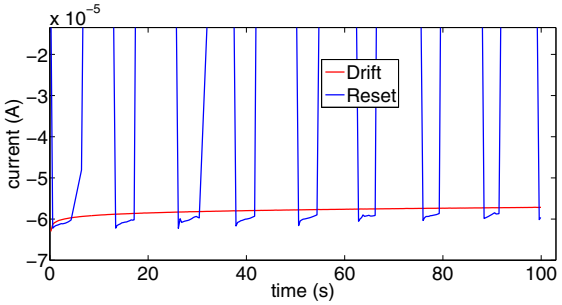

Fig. 4. The figure shows the current output of an ISFET, measuring buffer of pH 4, used in conjunction with a PWM circuit, showed in figure 3a, to reset the drift in the threshold voltage. The blue curve corresponds to the output of the ISFET while cycling the vertical electric field where as the red one correspond to the one where it is being operated continuously.

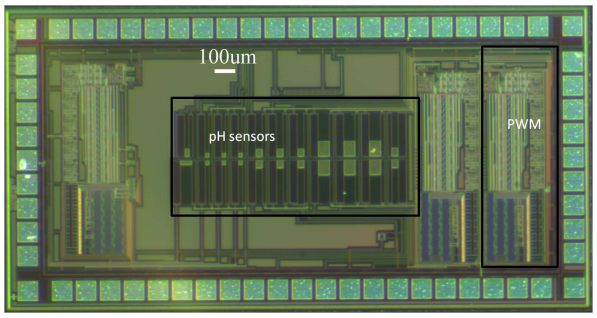

Fig. 5. Picture of the chip described in this work. The die was fabricated in a 0.5μm CMOS process. Also shown are the integrated pH sensors and the PWM circuit to be used with the sensors.

environment of the cells. This could lead to potential errors in the measurement. An integrated system composed of the PWM circuit designed in this work could be used in conjunction with an on chip sensors can be used for autonomous sensing. The addition of signal processing circuits, like the one in [19], for autonomous pH recordings inside the incubator, would compute contribution from temperature, drift, etc. This system allows us to continuously monitor cultured cells inside an incubator to determine behavior of caner cells and efficacy of therapeutic drugs. Future work includes creating an autonomous incubator [20], with the addition of a temperature sensor and feedback mechanism for maintaining the pH.

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