Integrated microfluidic device to monitor the cells acidification rate in nanoliter volumes using microfabricated iridium oxide electrodes

I. Ges, B. Ivanov and F. Baudenbacher

Vanderbilt University, Nashville, TN, USA, igor.ges@vanderbilt.edu

ABSTRACT

We describe a new approach to measure pH differences in microfluidic devices and demonstrate acidification rate measurements in on-chip cell culture systems with nanoliter wells. We used two miniaturized identical iridium oxide (IrO_x) thin film electrodes (20 x 400 μm^2), one as a quasi-reference electrode in the perfusion channel and the other as a sensing electrode in the cell culture volume. Our results show that we can use two identical miniaturized microfabricated IrO_x electrodes to measure pH differences and to monitor the metabolic activity of cell cultures on chip. Our approach is compatible with microfluidic technologies and allows for the fabrication of sensor arrays in combination with on-chip cell culture devices to implement a high throughput platform to monitor cell culture condition and the metabolic activity of a small number of cells.

Keywords: microfluidic device, pH measurement, iridium oxide

INTRODUCTION

Miniaturizing cell culture techniques to establish high throughput and high content drug screening platforms requires the incorporation of miniaturized sensor technologies to monitor the physiological condition of the cells on chip. Extracellular pH is a critical parameter for many cellular processes and extracellular acidification rates can be used to measure the metabolic activity of cells and to study the impact of toxins, chemicals and therapeutics on cell physiology. In order to record metabolic changes from a small number of cells in nano Liter (nL) volumes on chip it is necessary to scale down the sensor size and provide fluidic access to the cell culture volume for perfusion and toxin delivery. These requirements can be satisfied by developing planar thin film sensors with micron scale lateral dimensions on glass substrates, which act as a base layer for the microfluidic network fabricated using soft-lithography [1-3].

In our previous work [4] we described the design and fabrication of thin layer planar pH sensitive iridium oxide films with active sensing area below 500 μm^2 and demonstrated their performance by acidification rate measurements using macroscopic DRIREF-450 (WPI, Sarasota, FL) Ag/AgCl reference electrodes with a diameter of 0.45 mm inserted in the microchannel. However, the use of external reference electrodes causes a number of problems. The major problem is associated with the size of the reference electrode (0.15mm^2), which is comparable or larger than the working area of a microfluidic device (0.1mm^2). This requires a particular device topology with a compartment for the reference electrode, which has to be connected to the measurement volume through the channel network. This is especially difficult if a high level of fluidic control with valves is desired. Besides, it is necessary to provide a good seal between this electrode and the PDMS device. Therefore it is doubtful that macroscopic needle-type reference electrodes can be used in a mass-produced microfluidic-based cell culture device.

Thin film reference electrodes are the only feasible alternative to macroscopic reference electrodes. There are data in literature [5-7] demonstrating the use of thin film Ag/AgCl reference electrodes. Most of the thin film Ag/AgCl electrodes were fabricated using an electrochemical wet process. But the main problems of such electrodes are large potential drift (long-term stability), decomposition of AgCl in electrolyte solution, potential hysteresis during temperature cycles and cell toxicity [7]. As a result thin film Ag/AgCl reference electrodes are not suited to be used in microfluidic devices to monitor long-term metabolic rates and viability of living cells on chip.

The purpose of our research was to develop microfluidic-based devices with on-chip sensing to monitor the acidification rate of living cells using differential pH sensitive electrodes fabricated from electrochemically deposited iridium oxide thin films.

RESULTS AND DISCUSSIONS

The layout of the polydimethylsiloxane (PDMS) microfluidic device with overlaid sensing electrodes fabricated on a glass substrate is shown in Fig. 1a. The device has three microfluidic access ports: 1 – media input; 2 – waste output; 3 – cell loading port. The cell culture volume (working volume) is 9 nl (600 X 600 X 25 μm^3).

Thin films of IrO_x were formed using electrochemical deposition in the galvanostatic mode. The IrO_x layer properties and the detailed fabrication protocol are described in our previous work [4]. Anodically grown iridium oxide films used in this work showed Nernstain response with slopes ranging from - 55 ÷ - 65 mV/pH at 22^oC and linear responses within the pH range of 4-11. The response time of freshly deposited electrodes was 6-15 s and a typical baseline drift was 2-3 mV/day. We used four electrode designs for our experiments. IrO_x layers were deposited on Pt electrodes with dimensions of 20 x 400

μm^2. Uniform IrO_x layers (Fig.1b) were obtained utilizing a simultaneous deposition on all four electrodes in one single step.

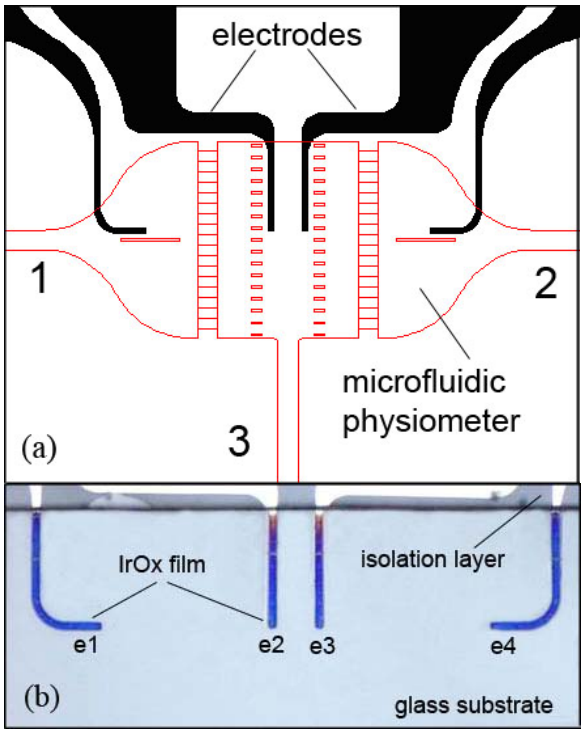

Fig.1. (a) Schematics of a microfluidic device with differential on-chip pH measurements; 1– input port; 2 – output port; 3 – cell load port. (b) Optical image of the iridium oxide microelectrodes (the width of electrodes is 20 μm). e1 and e4 – quasi-reference electrodes, e2 and e3 – pH sensitive electrodes.

The IrO_x electrodes were calibrated in three different buffer solutions versus an external CHI Ag/AgCl reference electrode. As shown in Fig.2, all four electrodes have practically identical electrochemical properties (sensitivity and response time). In the following experiments we used the electrodes e1 and e4 as a quasi-reference electrode and e2 and e3 as pH sensing electrodes.

The cells in the working volume are confined by two filters (Fig.1a), where the dimensions of the openings are – 3 μm (wide) X 25 μm (height) X 60 μm (length). The perfusion of cells in the microfluidic device is realized by applying pressure or vacuum to the input or the waste port, respectively. Our design is symmetrical and allows us to implement electrochemical measurements with multiple electrode pair combinations. After the cells were loaded into the working volume the cells were perfused through the input channel from left to right. The electrodes in the cell culture volume are used as sensing electrodes, whereas the electrodes in the input channel are exposed to cell culture media of a constant pH and are used as a quasi-reference electrode. The quasi-reference electrode is always in the buffer solution with constant pH, while the pH sensitive electrode is in the cell culture volume, in which we expect to sense pH changes due to the metabolic activity of the cells. In general, these measurements are possible in a stop flow mode, because there is no diffusion exchange between the effective fluid volumes of the electrodes due to the filter and the relatively short measurement intervals. Electrical connection between the pH and quasi-reference electrode takes place through the buffer solution in the device. Therefore, we measure the relative pH change which occurs in the cell culture volume as a result of the metabolic activity of the cells.

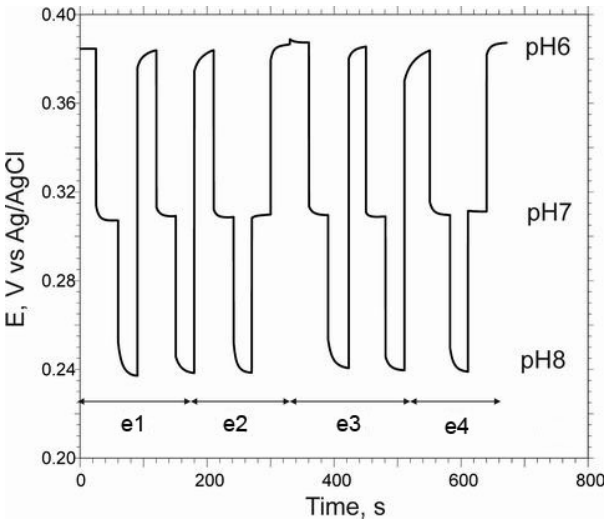

Fig. 2. Open circuit potential of e1– e4 IrO_x pH electrodes during periodic cycling of 3 different standard pH calibration solutions versus external CHI Ag/AgCl reference electrode. The experiments were performed in a beaker with 30 s measurement intervals.

In order to validate the sensitivities of the IrO_x electrodes in a differential measurement arrangement using on-chip reference electrodes, we have designed a Y-shaped simple microfluidic device to implement a simple calibration protocol. Electrode e1 was situated in channel M and electrode e4 in channel N (Fig. 3d). The calibration buffer solutions were colored with pH neutral food colors to visualize the flow conditions and to distinguish solutions with different pH under the microscope. For our experiment we used two buffer solutions, one with pH=7 colored in red and the other with pH=8 colored in blue (Fig. 3b, c). A pH difference could be established by changing the relative flow rates in the input channels. No potential difference between the electrodes was observed when both channels were filled with the same buffer solution (Fig. 3d). The maximum potential difference of ~65 mV was obtained with a buffer solution of pH=7 in channel M and buffer solution of pH=8 in channel N. These data agree (Fig.2) with the results of our initial pH calibrations using an external standard Ag/AgCl electrode. An intermediate state could be achieved by adjusting the flow ratios between the

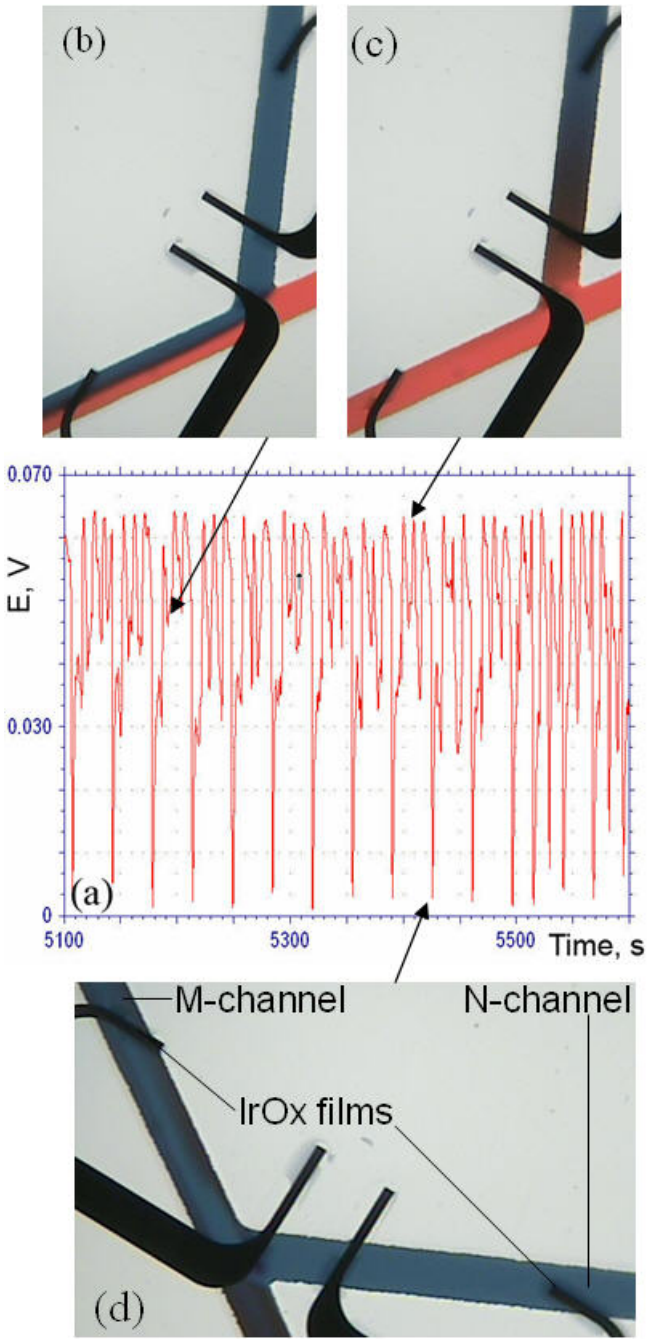

Fig. 3: Validation of a differential pH measurement scheme using two IrO_x electrodes and a Y-shaped microfluidic device to expose each electrode to solutions of different pH. (a) Open circuit potential measurements between e4 (quasi-reference) and e1 (pH) IrO_x electrodes during periodic cycling of two colored buffer solutions (pH7 and pH8); (b-d) Optical images of flow patterns in the Y-channel above the IrO_x pH sensing and quasi-reference electrode; (b) Channel N filled with pH8 solution and laminar flow pattern in the channel M. The pH sensing electrode is simultaneously exposed to both pH7 and pH8 solutions; (c) Channel M filled with pH7 and channel N filled with pH8 solutions; (d) Channels M and N filled with the same pH8 solution.

two input channels, which typically results in flow oscillations as shown in Fig. 3a. The potential differences correspond to different flow patterns where we observed laminar flow of two solutions with different pH (Fig. 3b) in channel M. Thus, these experiments validate our approach of using two identical IrO_x sensors to measure pH differences in a microfluidic environment and show that the sensitivity is not changed compared to our initial calibration using standard Ag/AgCl reference electrodes.

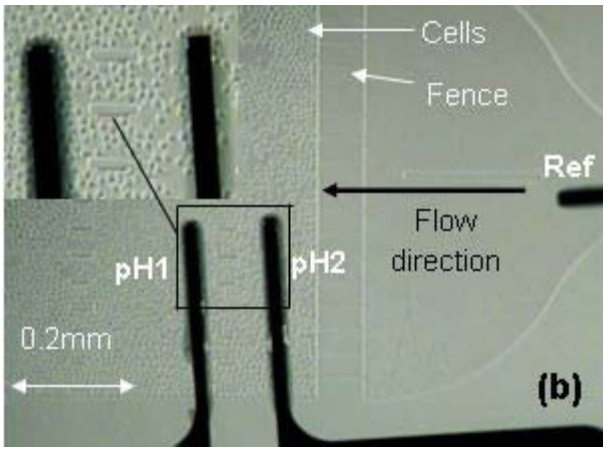

Fig.4: Optical image of the pH sensing and quasi-reference iridium oxide electrodes inside the 9 nL cell culture volume filled with fibroblast cells during acidification measurements. The inset shows a close view of the sensing volume around pH microelectrodes.

Subsequently, we used IrO_x electrodes in a differential pH measurement configuration to quantify the acidification rate of fibroblast cells in the cell culture volume. Fig. 4 shows an optical microscopic view of the on-chip cell culture device, with working volume of 600µm X 600µm X 25µm, loaded with fibroblast cells. In the working volume there are two pH sensitive electrodes (pH1 and pH2). The IrO_x quasi-reference electrode was situated in an input perfusion channel (Fig. 4). The perfusion direction was always maintained, allowing the use of an IrO_x quasi-reference electrode exposed to the culture media. The sensing electrode is positioned in the cell culture volume, where the metabolic activity of the cells leads to acidification of the media. In such a way the reference electrode always is exposed to the solution with known and constant pH. The cell culture volume was typically loaded with fibroblast cells to about 60-85% of full capacity by gravitational feeding. Fig. 5 shows time traces of the voltage difference between sensing and the quasi-reference electrode in the stop-flow mode using our differential pH measurement configuration. After stopping the flow and a delay we observe a change in extracellular pH which was

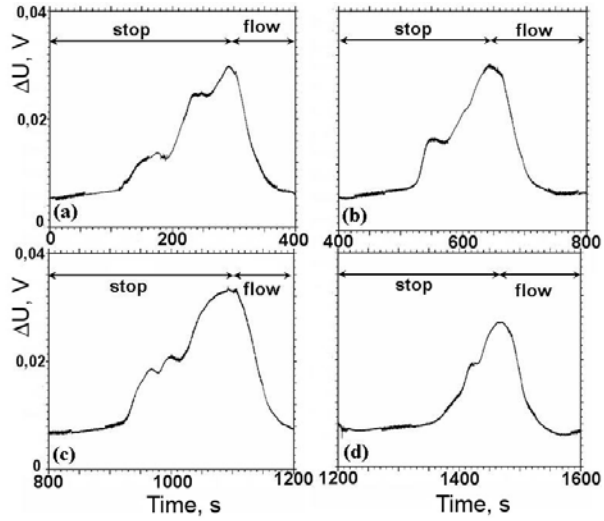

Fig. 5. Open circuit potential between IrO_x pH sensing and the IrO_x quasi-reference electrode using a stop-flow protocol for acidification rate measurements of fibroblasts in the 9 nL cell culture volume. The perfusion rate was 2 nl/s.

reversible after switching into the flow mode. The signal always returned to the initial base line. Small differences in the heights and shape of the peaks we attribute to flow induced redistribution of the cells around the measuring electrode and possibly fluctuation of the perfusion flow.

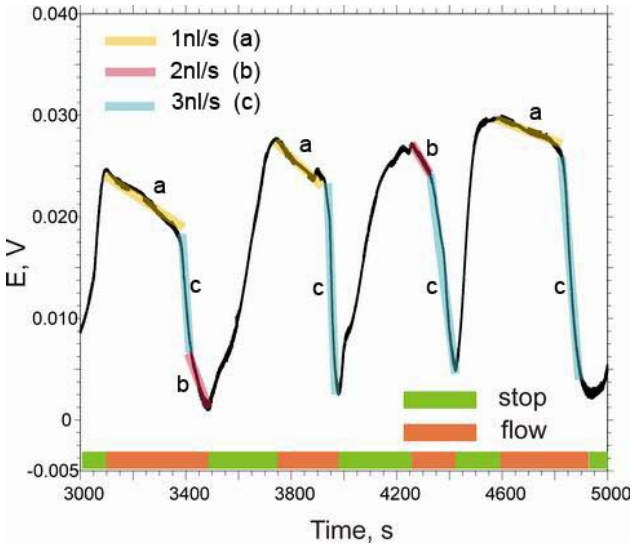

Fig. 6. Open circuit potential differential pH measurements of fibroblast cells' acidification at stop-flow mode for three different flows at 1, 2, and 3 nl/s in a microfluidic device.

Based on our measurements we calculated the acidification rate of these cells to be 0.03 pH/min. This result agrees with our previous measurements using external Ag/AgCl reference electrodes [4]. In general, the measured acidification rate depends on the metabolic activity of the cells and the perfusion rate. We confirmed this experimentally by varying the reperfusion rate in the range 1-3 nL/s. In Fig. 6 we presented the potential difference between quasi-reference and pH sensitive iridium oxide electrodes for 4 cycles switching from the stop to flow mode. We observed characteristic slopes depending on the reperfusion rate. In the future we plan to adjust the perfusion rate to maintain a constant pH to maintain homeostasis. The required perfusion rate would therefore be a measure of the metabolic activity.

CONCLUSION

We introduced a new approach to measure pH differences in microfluidic devices. We used IrO_x thin films both as pH sensitive and quasi-reference electrodes. We confirmed experimentally that our electrode sensitivity exactly corresponds to the electrode sensitivity obtained using an external Ag/AgCl reference electrode when the two thin film electrodes are exposed to a pH difference of one unit and referenced to each other. We also demonstrated on-chip pH sensing by measuring the acidification rate of fibroblast cells confined in an on-chip cell culture volume. Using IrO_x thin films as a quasi-reference electrode substantially simplifies technological processes for pH sensor formation. Additional advantages of IrO_x layers are their biocompatibility and their long-term stability when exposed to cell culture media.

REFERENCES

[1] H.Andersson, A. van den Berg, "Microfluidic devices for cellomics: a review," Sensors and Actuators B, **92** (3), 315-325, 2003.

[2] A.R. Wheeler, W.R Throndset, et al., "Microfluidic device for single-cell analysis," Analytical Chemistry, **75** (14), 3581-3586, 2003.

[3] A. Prokop,Z. Prokop,et al., "NanoLiterBioReactor: Long-Term Mammalian Cell Culture at Nanofabricated Scale," Biomedical Microdevices, **6**, 325-339, 2004

[4] I.A. Ges, B.L. Ivanov et al., "Thin-film IrOx pH microelectrode for microfluidic-based Microsystems," Biosensors & Bioelectronics, **21** (2), 248-256, 2005.

[5] A. Hiratsuka, K. Kojima, et al., "Integration of microfabricated needle-type glucose sensor devices with a novel thin-film Ag/AgCl electrode and plasma-polymerized thin film: mass production techniques", Analyst, **126 (**5), 658-663, 2001.

[6] A. Simonis, H. Luth, J. Wang, M. J. Schoning, "Strategies of miniaturised reference electrodes integrated in a silicon based "one chip" pH sensor", Sensors, **3 (**9), 330-339, 2003.

[7] T. Matsumoto, A. Ohashi, N. Ito, Analytica Chimica Acta, , **462,** 253-259, 2002.