Investigation of pH behavior of single myocyte cells in picoliter microfluidic device

Igor A. Ges, Franz J. Baudenbacher

Department of Biomedical Engineering, Vanderbilt University, 5824 Stevenson Center, VU Station B 351631, Nashville, TN 37235-1631, USA; E-mail: igor.ges@vanderbilt.edu

ABSTRACT

We have combined a microfluidic network and miniature differential thin film pH sensors to measure acidification rates of single cardiomyocytes in a confined extracellular space. The thin film IrO_x pH sensors were combined with stimulating microelectrodes and integrated the 160 picoliter extracellular space. We used two miniaturized identical iridium oxide (IrOx) thin film electrodes (40x80 μm^2), one as a quasi-reference electrode, and the other as pH sensing electrode, placed in two confluent compartments of microfludic device. Single cardiac cells were trapped in the sensing volume using pressure gradients. Using miniature mechanical valves allowed us eliminate the influence of flow due to residual pressure gradients on the open circuit potential. Elimination of flow artifacts resulted in reproducible measurements of acidification rate of single cardiac myocyte cells in a confined extracellular space. In accordance to our experiment the average acidification rate was $7x10^{-3}$ pH/min for wild type and $19x10^{-3}$ pH/min for VLCAD type of cardiac myocytes.

Keywords: microfluidic device, pH measurement, iridium oxide films, cardiomyocytes, acidification rate.

INTRODUCTION

Microfluidic devices are becoming wide spread in different areas of biology and medicine [1-6]. These devices range from disposable point of care devices to miniature highly parallel cell culture devices. Although, progress has been made incorporating sensors into lab-on-a-chip devices there is a lack of high density sensor arrays. Small sensor configurations are necessary to measure pH changes in vicinity of single cells. Measuring the acidification rate will allow us to characterize the metabolic activity of single cells in response to pharmacological and toxicological interventions. Investigating single cell metabolic activity in real time in sub-nanoliter volumes could eventually constitute a more realistic in vivo like chemically controlled microenvironment compared to culture dishes and provide novel insights into fast physiological processes. To implement acidification rate measurements we have developed a microfluidic device with active valves, which confines single cardiac myocytes in a 160 pL volume on microfabricated planar thin film pH electrodes. This sensing approach was used to measure acidification rates in a mouse phenotype with Very Long Chain Acetyl-CoA Dehydrogenase (VLCAD) deficiency preventing beta-oxidation of fatty acids. Due to the lack of VLCAD the glycolytic pathway is impaired and one would expect an increase in lactic acid production and therefore acidification rate.

RESULTS AND DISCUSSIONS

Fig. 1a shows the design of our microfluidic device and the layout of our sensing and stimulation electrodes. The microfluidic device was fabricated using soft lithography techniques and polydimethylsiloxane (PDMS) replication molding. The electrodes are deposited and patterned on glass substrates. The device has five microfluidic access ports: 1 – cell loading port; 2 – waste output; 3 – vacuum input for generating a pressure gradient to trap single cells; 4, 5 – drug input channels (stimulate electrodes channels). The volume of the extracellular space is 0.16 nL (200 X 40 X 20 μm^3). A pressure gradient was manually applied to trap single cell from input port into the sensing volume. Two designs of orthogonal stimuli Pt electrodes were developed. With this electrode configuration, we were able to apply electrical field either perpendicular (Fig.1b) or parallel (Fig. 2a) to the longitudinal axis of cardiac myocytes.

The base metal electrode structures were evaporated on glass microscope slides (25mm×25mm×1 mm). These electrodes consisted of two metal layers: a Ti adhesive layer (10 nm) and a Pt working layer (100 nm). Thin-film electrodes were deposited by e-beam vacuum evaporation of Ti and Pt from carbon crucibles. The Ti and Pt films were evaporated in a single process without breaking the vacuum. The metal films were patterned using standard photolithography processes and ion etching to remove the metal in all areas not protected by photoresist.

IrO_x films were selectively electrodeposited onto the unprotected microfabricated Pt electrodes in the galvanostatic mode. The IrO_x layer properties and the detailed fabrication protocol are described in our previous work [7]. Anodically grown iridium oxide films used in this work showed Nernstain response with slopes ranging from 65 ÷ 75 mV/pH at 22°C 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

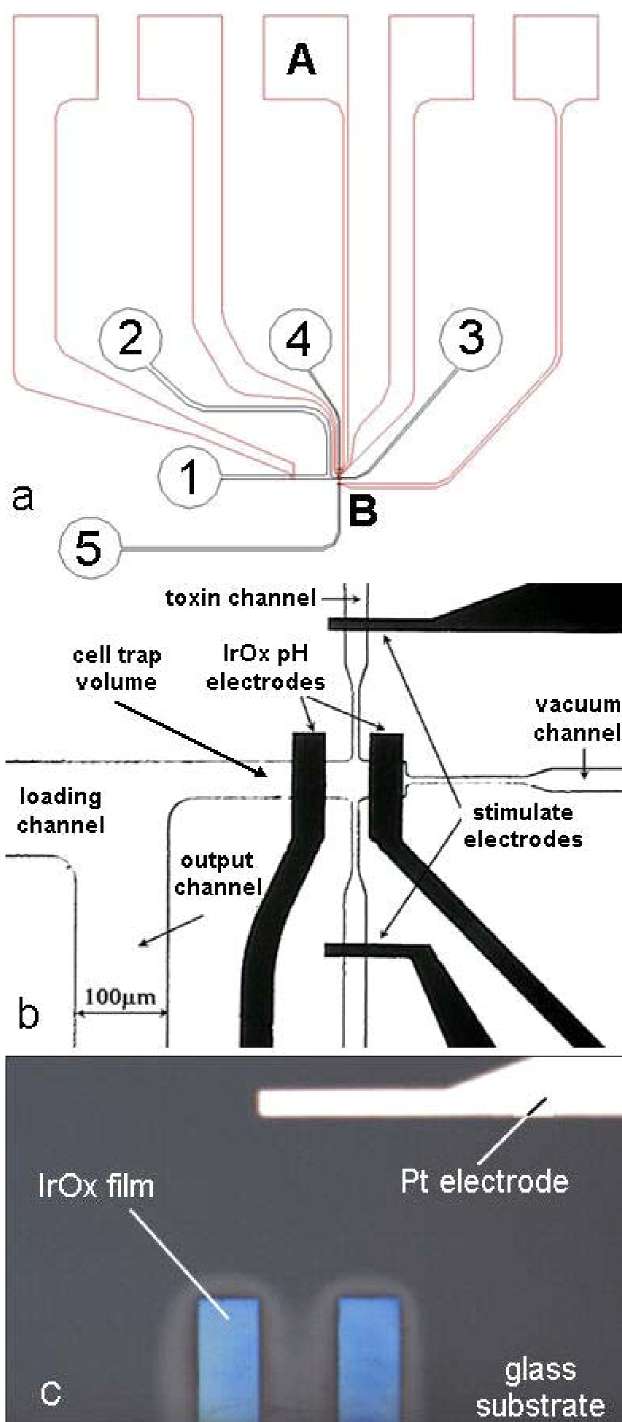

Fig.1.(a) Schematics of a microfluidic device with differential pH measurements of single cardiac myocyte cell; microelectrodes array (A), PDMS microfluidic device (B).(b)Detailed view of pH IrOx microelectrodes in the cell trap volume and the perfusion channels. (c) Optical image of the IrOx microelectrodes (the width of electrodes is 40 μm).

mV/day. We used two different three IrOx electrode designs for our experiments (Fig. 1b and Fig. 2a). IrO_x layers were deposited on Pt electrodes with dimensions of 40 x 200 and 20 x 400 μm^2 . Uniform IrO_x layers, essential for differential sensing, (Fig.1c and Fig. 2b) were obtained utilizing a simultaneous deposition on all three electrodes in one single step.

In our differential sensing configuration, we used IrO_x thin films both as pH sensitive and quasi-reference electrodes [8]. We confirmed experimentally that our electrode sensitivity corresponds exactly to the 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. One IrOx pH sensitive electrode was situated in the cell trap in close proximity to the cardiac cell and the other IrOx quasi-reference electrodes was located in the loading channel. Using IrO_x thin films as a quasi-reference electrode substantially simplifies the implementation and stability of pH sensor configurations in a microfluidic environment. Additional advantages of IrO_x sensors are their long-term stability and their biocompatibility when exposed to cell culture media.

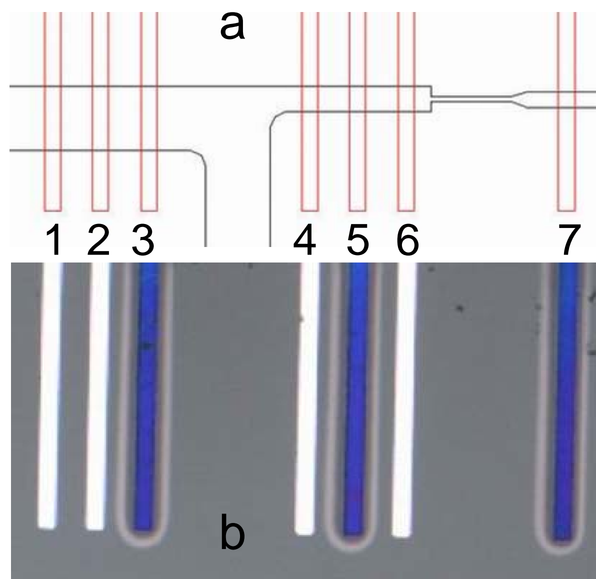

Fig.2.(a) Schematic of a microfluidic device for electrical stimulation of cardiac myocyte cell: black – configuration of channels in the working part of PDMS microfluidic device; red – microelectrodes. (b) Optical image of microelectrodes (the width of electrodes is 20 μm). 5 - IrOx pH sensitive electrode; 3, 7 - IrOx quazi reference electrodes; 4, 5 – Pt electrical stimuli electrodes

In order to measure acidification rates and characterize the metabolic activity of cells the flow in microfluidic devices needs to be controlled very precisely. In microfluidic-based platforms there are a wide range of fabrication strategies for valves and switches to control the flow of fluids and delivering of specific amounts of reagents. Even small leak rates can be detrimental therefore

we decided to implement miniature mechanical valves. Our design allowed us to place three valves in close proximity to the cell trap within a footprint of 6x8 mm^2 (Fig.2a).The valves were fabricated by drilling a pocket hole above the microfluidic channel into the PDMS. Into the pocket hole we inserted an oversized threaded sleeve and a screw which allows us to compress the PDMS in order to close the microfludic channel completely. Fig.2 shows the operation of the mechanical valve. The functionality and the leak rate of the valves were demonstrated with a dye solution. Fig. 2b shows the channel open and the bottom image shows the channel closed after rotating the screw is shown on fig.2c. The use of valves proofed to be essential in obtaining reliable results of acidification rate measurements of single cardiac myocyte cells since small pressure gradients result in unpredictable flow patterns in microfluidic devices.

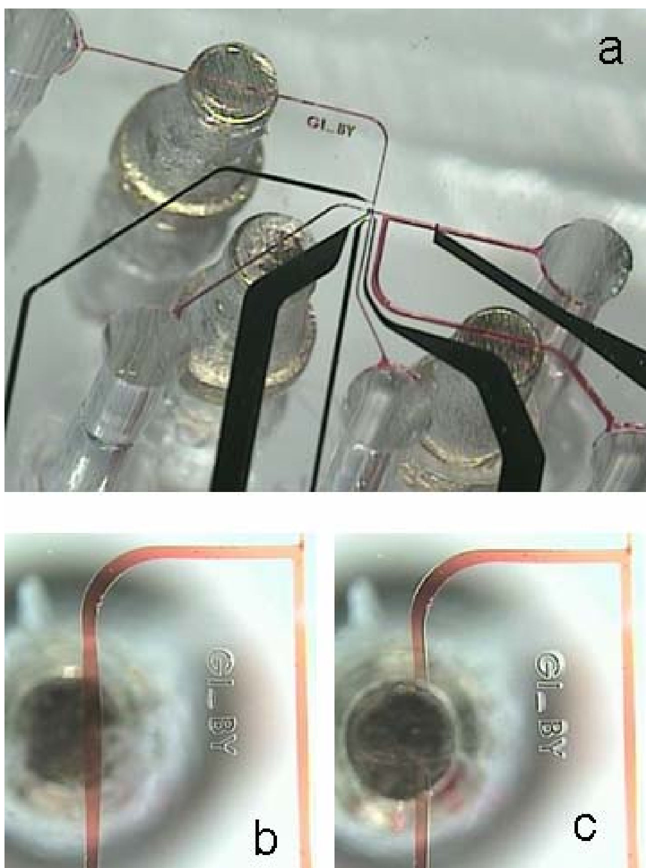

Fig.3. Images of mechanical valves. (a) Microfluidic device with 3 valves (black – Pt electrodes; red – microfluidic channels: 100 μm wide, 20 μm tall). (b) Bottom view of a channel filled with food dye; the valve is open. (c) Bottom view of a channel that has been completely closed by rotating the screw.

The microfluidic network as attached to the glass substrate with the pacing and sensing electrodes through auto adhesion. Therefore the device could be used multiple times and the microelectrodes array matched to different PDMS microfluidic structures. A cell suspension was loaded into the microfluidic device through input port 1 (fig 1a). After priming a negative pressure was applied to the output (2) to pull single cells from input port into close proximity of the trap. Then a brief negative pressure is applied to the cell trapping channel moving the cell into the sensing volume. By reversing the pressure in the cell trapping channel the cell could be removed from the trap. Once the cells are trapped we closed the channels leading to the sensing volume using our simple mechanical screw valve design (fig 3a, b, c). Since two IrOx pH electrodes were directly incorporated in the cell trap volume we could select different electrodes to compensate for difference in the size of cardiomyocytes. The IrOx reference electrode was located in the cell loading channel at a distance of 500 μm from the cell trap. Details of the differential pH sensing approach are described elsewhere [8].

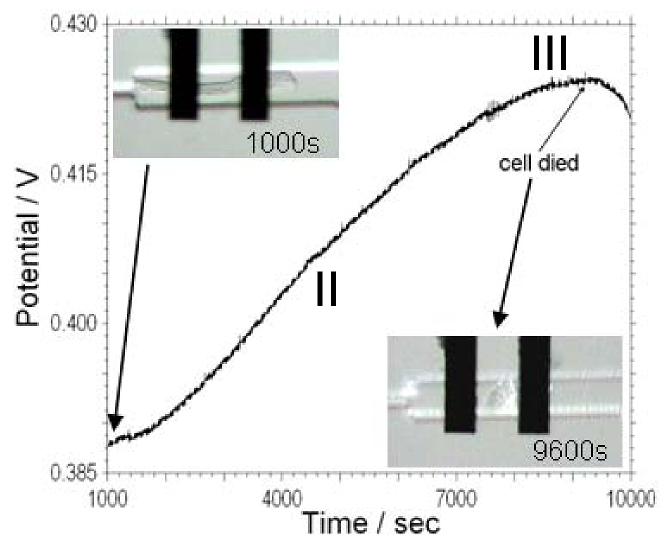

Fig.4. Open circuit potential between IrOx pH sensing and the IrOx quasi-reference electrode for acidification rate measurements of cardiac myocyte single cell. The inset show a detailed view of cardiac myocyte cell in the cell trap volume in the initial and the final stage of experiment.

The metabolic activity and the physiological state of living cells can be characterized by the rate at which the cells generate acidic by-products. We conducted our experiments on murine cardiac myocytes VLCAD and compare these to wild type. Figure 4 shows a typical time course of the open circuit potential of the IrO*x* pH microelectrode during an acidification rate measurement on a single myocyte in the cell trap. There are three regions that can be characterized by different acidification rates (Fig.4). After closing of the valves there is a region of 20-60 second duration characterized a large drift in the open circuit potential most likely associate with residual flow in the microfluidic device. The second region (10 – 90min) is characterized by a significantly lower acidification rates. In third region is characterized by a very low acidification rate

NSTI-Nanotech 2007, www.nsti.org, ISBN 1420061836 Vol. 2, 2007

indicating a lower metabolic activity which could be caused by low pH of the cellular environment. In order to avoid these large deviations from homeostasis we analyzed the acidification rate of different type of cardiac myocytes in the second region. We measured an average acidification rate for wild type cardiomyocytes of ~ 6.45 mpH\min and ~19.5 mpH\min for VLCAD.

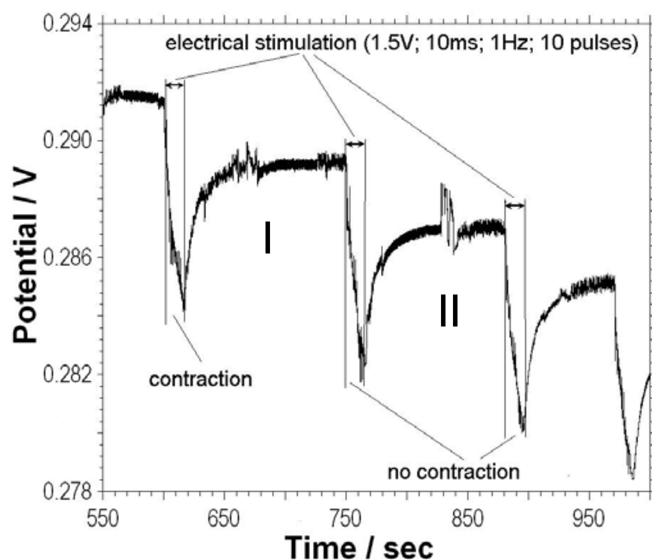

Fig.5. Open circuit potential of differential pH measurement of cardiac myocyte single cell during electrical stimulation (parameters of electrical pulses: amplitude 1.5V; duration 10 ms; frequency 1Hz).

The second goal of our research was to explore the effects of electrical stimulation on acidification rates of cardiac myocytes. One of the serious problems in this kind of measurements is that the electrical impulse causes a shift in the sensing electrode potential that in turn complicates the interpretation of the results. Figure 5 shows the pH electrode potential during a train of 10 1.5V 10 ms long electrical pulses used for stimulation. In region I we observed contractions initiated by the field potential generated by the stimulation electrodes. In region II the cell did not contract when exposed to electric fields. Analyzing and superimposing the data showed that the regions were identical. Furthermore, changing the polarity of the stimulation pulses as well as implementing a bidirectional stimulation protocol or changing the direction of the stimulation field relative to the pH sensing electrodes did not change the measured potentials. Therefore we could conclude that electrical stimulation and contraction didn't affect acidification rates. This interpretation is consistent with results by Cooper et al [6] using fluorescent techniques to measure the extracellular pH.

In conclusion, we have demonstrated the feasibility of measuring acidification rates of single cardiac myocytes in an enclosed confined extracellular volume using a differential sensing approach utilizing IrOx electrodes. Further development of this platform with addition of other types of sensors (glucose, lactose, and oxygen) and the incorporation of optical and fluorescent methods allow the simultaneous recording of multiple physiological variables in real time.

ACKNOWLEDGMENTS

We especially thank Dr. Igor Dzura for culturing cardiac myocyte cells and helpful discussions.

REFERENCES

[1] D. Carlo, L. Wu L. Lee Dynamic single cell culture array. *Lab Chip*, 2006, **6**, 1445-1449.

[2] C. Bratten, P. Cobbold, J. Cooper. Single-Cell Measurements of Purine Release Using a Micro-machined Electroanalytical Sensor. *Anal. Chem*. 1998, 70, 1164-1170.

[3] C. Bratten, P. Cobbold, J. Cooper. Micromachining Sensors for Electrochemical Measurement in Subnanoliter Volumes. *Anal. Chem.*, 69 (2), 253 -258, 1997.

[4] X. Cai, N. Klauke, A. Glidle, P. Cobbold, G. Smith, J. Cooper. Ultra-Low-Volume, Real-Time Measurements of Lactate from the Single Heart Cell Using Microsystems Technology. *Anal. Chem*. 2002, 74, 908-914.

[5] A. Werdich, E. Lima, B., I. Ges, M. Anderson,J. Wikswo, F. Baudenbacher. A microfluidic device to confine a single cardiac myocyte in a sub-nanoliter volume on planar microelectrodes for extracellular potential recordings.*Lab Chip* , 2004 , 4 , 357 – 362.

[6] W. Cheng, N. Klauke, H. Sedgwick, G. L. Smith, J. M. Cooper. Metabolic monitoring of the electrically stimulated single heart cell within a microfluidic platform. *Lab Chip*, 2006.

[7] I.A. Ges, B.L. Ivanov et.al. Thin film IrOx microelectrodes for microfluidic based Microsystems. Biosensors and Bioelectronics, 21 (2005) 248-256.

[8] I. Ges, B. Ivanov, A.Werdich, F. Baudenbacher. Differential pH measurements of metabolic cellular activity in nL culture volumes using microfabricated iridium oxide electrode. *Biosensors and Bioelectronics* 22 (2007) 1303–1310.