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Ruthenium Oxide pH Sensing for Organs-On-Chip Studies †

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Abstract: A ruthenium oxide (RuOx) electrode is being developed as potentiometric pH sensor for organs-on-chip applications. Open-circuit potential (OCP) of the RuOx electrode showed a response of −58.05 mV/pH, with no cross-sensitivity to potentially interfering/complexing ions (tested were lithium, sulfate, chloride, and calcium ions). Similar response was observed in complex biological medium. The electrode stored in liquid had a long-term drift of −0.8 mV/hour (corresponding to ΔpH of 0.013/hour) and response time in complex biological medium was 3.7 s. Minimum cross-sensitivity to oxygen was observed as the OCP shifted ~3 mV going from deoxygenated to oxygenated solution. This response is one magnitude lower than previously reported for metal-oxide pH sensors. Overall, the RuOx pH sensor has proven to be a suitable pH sensor for organs-on-chip applications.

Keywords: ruthenium oxide; potentiometric sensor; pH sensor; organs-on-chip; lab-on-chip

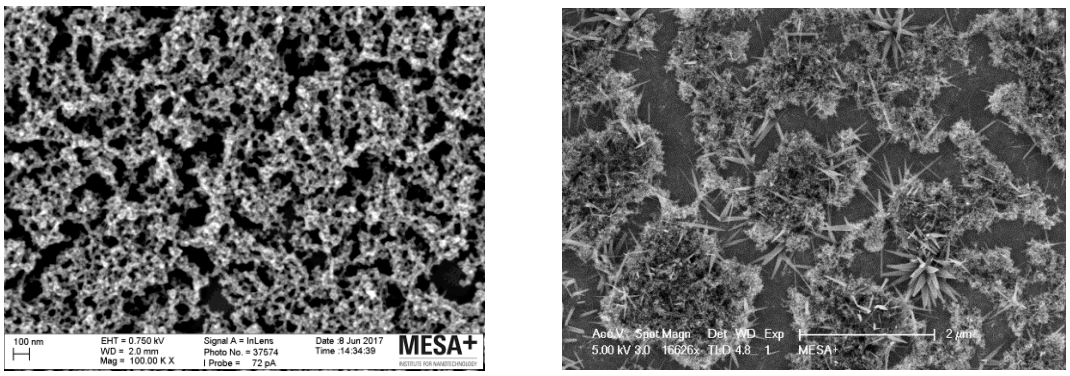
1. Introduction

Organs-on-chips are in vitro models of human tissue, where cells are cultured inside microfluidic devices, allowing accurate mimicry of cell's microenvironment. However, it also leaves low accessibility to monitor the cells from outside the chip. Microsensors are therefore an invaluable option in providing read-out from within the devices, as they can be placed in close proximity to the cells, enabling accurate online monitoring of minute analytes. This work explores on-chip pH sensors, which are useful for long-term cell-culture and tissue studies. Metal oxide (MeOx) is a well-known class of on-chip solid-state pH sensors, for its robust, inert, and miniaturizable properties. However, they often suffer from oxygen cross-sensitivity [1,2], a drawback for organs-on-chip applications. Ruthenium oxide (RuOx), notably, is an exception to this shortcoming.

RuOx's pH sensitivity is based on the redox equilibrium between two different solid phases of RuOx, in which a proton is involved [3–5]:

$$RuO_2 \cdot 2H_2O + H^+ + e^- \leftrightarrow Ru(OH)_3 + H_2O \quad (1)$$

For this work, a RuOx electrode is fabricated from $Ru(OH)_3$ precursor (Figure 1a), which formed RuO_2 nanorods (Figure 1b) after heat treatment. The RuOx electrode is intended to be later developed into a dual sensor of potentiometric pH sensor and amperometric nitric oxide sensor, where the nanorods morphology is paramount for the latter. Therefore, the same RuOx nanorods are studied as pH sensor, although the morphology does not theoretically improve the potentiometric signal. This contribution presents the RuOx pH sensing characteristics as well as initial results on the work towards first application of the RuOx on-chip pH sensor.



(**a**) (**b**)

Figure 1. SEM images of the RuOx electrode: (**a**) Precipitated $Ru(OH)_3$ precursor on silicon surface; (**b**) Heat-treated precursor (at 350 °C, for 3 h) on platinum formed RuO_2 nanorods with width of around 50 nm and length of 200–350 nm.

2. Materials and Methods

2.1. Electrode Fabrication

The RuOx electrode was fabricated following the protocol of Chen et al. [6] $RuCl_3.xH_2O$ was dissolved in de-ionized water to make 5 mM solution. 5 mM NaOH solution was added until precipitation occurred. The precipitated $Ru(OH)_3$ precursor was isolated and resuspended in DI water. The resuspension was then spread on a clean substrate (sputtered circular Pt electrode, 2.4 mm in diameter, on glass chip) and heated to 350 °C for 3 h. RuO_2 nanorods were formed after the heat treatment (verified by SEM imaging).

2.2. Experimental Setup

The modified chip with grown RuOx nanorods was used for all experiments. The glass chip was used with an in-house made Teflon chip holder. All potentials were measured against a liquid-junction Ag/AgCl (satd. KCl) reference electrode (CH Instruments). Measurements were carried out using Bio-Logic SP300 bipotentiostat (input impedance of >100 GΩ) and were performed at 22 °C. The pH of the solutions was confirmed with a Mettler-Toledo SevenMulti pH meter.

3. Results and Discussion

Figure 1 compares the freshly precipitated $Ru(OH)_3$ precursor (Figure 1a) and the typical RuO_2 nanorods on cleaned Pt electrode produced by heat treatment (Figure 1b).

Typical pH response of the RuOx electrodes is shown in Figure 2a. Linear fitting shows a pH sensitivity of -58.05 mV/pH and extrapolated E^0 of 0.736 V. These figures come in good agreement with predicted theoretical values given in Equation 2 [3,5], thus affirming the pH sensing theory.

$$E = -0.059\ \text{V} \cdot \text{pH} + 0.740\ \text{V (vs. Ag/AgCl)} \quad (2)$$

MeOx can be readily complexed by different (an-)ions [1,5]. Application in biological milieu will inevitably expose the RuOx electrode to possibly complexing ions. Therefore its response towards lithium, sulfate, chloride, and calcium was studied. Minimum response was found towards the ions. Highest sensitivity, towards lithium ions at 1.06 mV/decade [Li^+], is also plotted in Figure 2a as comparison to the pH response.

A closer study into RuOx performance in complex biological medium is shown in Figure 2b. RuOx OCP was measured in cardiomyocytes medium (pH 6–8) and plotted against measurements by commercial pH meter. Linear fitting showed slightly poorer pH sensitivity of −56.89 mV/pH. Some interactions between species in the medium and the RuOx electrode could result in this lower sensitivity. However, the same interactions could also occur on the commercial pH electrode, introducing error to the measurements. Calibration of RuOx in pH buffers (pH 2–10) after prolonged

exposure to the cardiomyocytes medium showed the original sensitivity of −57.97 mV/pH, indicating no irreversible biofouling occurred from interaction with complex biological medium.

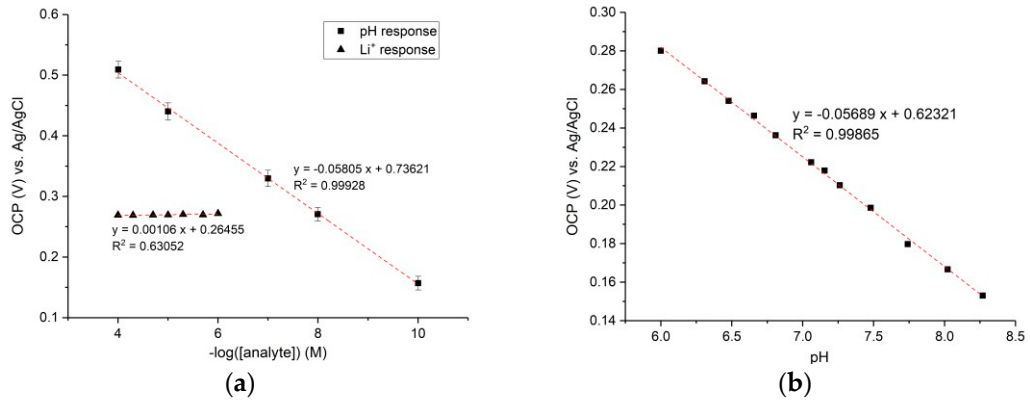


Figure 2. (**a**) RuOx OCP in pH buffer solutions. Error bar shows one standard deviation out of three different measurement sets. Largest cross-sensitivity (towards Li^+) is also plotted; (**b**) RuOx OCP in cardiomyocytes medium plotted against pH measured by commercial pH meter (Mettler-Toledo).

Drift behavior of the RuOx electrode was studied by recording OCP of the RuOx electrode in air-saturated pH 7 buffer. Firstly, after the electrode had been stored in air (Figure 3a, dry-stored RuOx), then after it had been stored in pH 7 buffer overnight (Figure 3a, wet-stored RuOx). Dry-stored electrode showed high drift of −7.5 mV/hour, while wet-stored RuOx electrode showed significantly lower drift of −0.8 mV/hour. The wet-stored drift corresponds to ΔpH of 0.013 per hour, which can be sufficient for measurements over several hours in organs-on-chip applications.

Time response of the RuOx electrode in complex biological medium can be seen in Figure 3b. RuOx OCP was recorded in stirred cardiomyocytes medium, during which the medium pH was changed by additions of lactic acid. Analysis of the slopes results in an estimated response time around 3.7 s, a sufficiently fast response for the foreseen organs-on-chip applications.

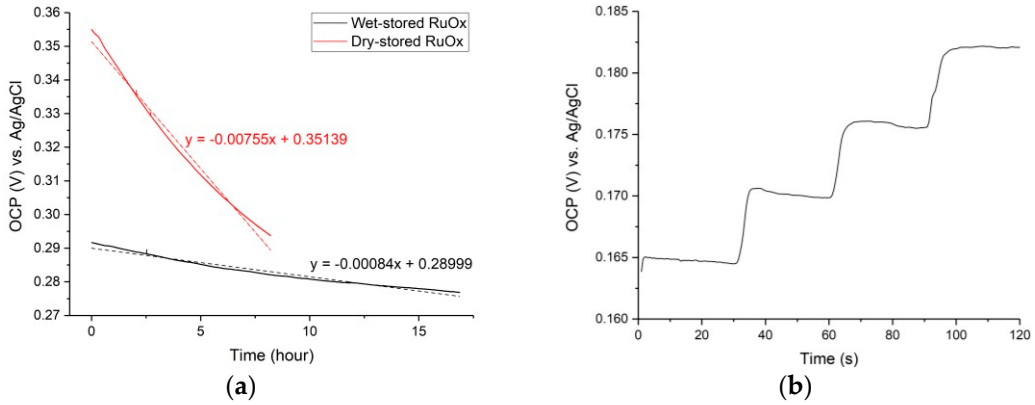


Figure 3. (**a**) RuOx OCP recorded over time in pH 7 buffer, fitted linearly (dashed lines). RuOx electrode showed high drift when stored in air (dry-stored) and significantly lower drift when stored in liquid (wet-stored); (**b**) RuOx OCP recording in stirred cardiomyocytes medium, pH was changed by additions of lactic acid.

RuOx OCP was also recorded in changing oxygen concentration (Figure 4). The OCP recording in deaerated buffer shows a negatively sloping potential (the first 4 h, linearly fitted with blue dashed line) of the same order of magnitude as the earlier observed wet-stored drift. A positive slope was recorded in the following four hours as reoxygenation took place (linearly fitted with red dashed line). Over the entire 4 h, the OCP drifted 3 mV as the buffer went from deoxygenated to oxygenated. This response proved to be significantly lower than other reported MeOx sensors (which can have response as much as 80 mV) [2]. The 3 mV potential shift corresponds to ΔpH of 0.050. Since oxygen level in

organs-on-chip applications will either be stable or roughly known, a careful characterization/calibration of the potential-pH response in such environment can ensure an accurate online monitoring of pH.

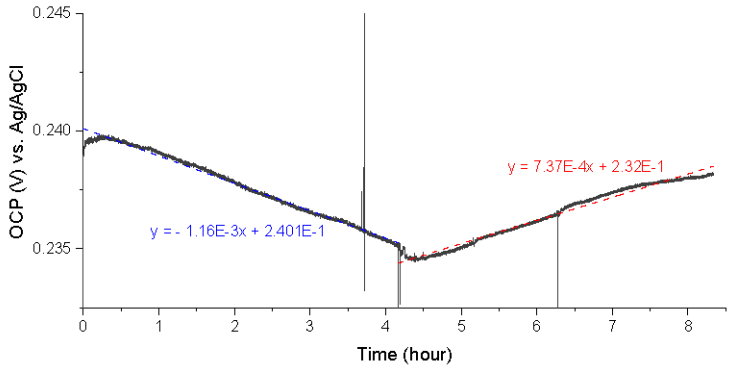


Figure 4. OCP recording of wet-stored RuOx electrode, for the first 4 h in deaerated pH 7 buffer, followed by 4 h of reoxygenated pH 7 buffer. Fitted linearly by the dashed lines.

4. Conclusions and Outlook

A pH sensitive RuOx nanorods electrode was successfully fabricated by a simple heat-treatment of Ru(OH)3 precursor. The OCP of the electrode showed a near-Nernstian response to pH, with high selectivity against potentially interfering ions. The electrode proves to be a suitable pH sensor for future organs-on-chip applications, also in terms of drift, response time, and oxygen sensitivity. Future work includes application of the RuOx nanorods pH sensor in hypoxic cardiomyocytes study. Cardiomyocytes switch to anaerobic metabolism under hypoxia, characterized by a drop in their extracellular pH. pH monitoring of this process can elucidate different pathways in this tissue study. Furthermore, the same RuOx nanorods is also being studied for its amperometric nitric oxide sensing.

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Conflicts of Interest: The authors declare no conflict of interest.

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