MICROFLUIDIC REFERENCE ELECTRODES FOR APPLICATIONS IN BIOSENSING

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ABSTRACT

A microfluidic Silver/Silver Chloride (Ag/AgCl) reference electrode was fabricated and operated in various modes. This micro-scale reference electrode design includes all the features of the macro-scale version including, Ag/AgCl electrode, inner standard solution and nanoporous membrane separator in a small form factor. This miniature reference electrode could also be operated in modes where the liquid junction potential can be stabilized to provide a stable potential. In one such mode, the microfluidic reference electrode with a free diffusion liquid junction shows < 0.1 mV stability over a 100-hour lifespan and no sensitivity to pH and chlorine concentration of the external solution.

KEYWORDS: Ag/AgCl reference electrode, free-diffusion liquid junction, microfabrication, microfluidics

INTRODUCTION

Reference electrodes are used in situations where electrical sensors interface with ionic solutions and are typically used to impose or measure an electric potential in a solution [1]. Reference electrodes are used in many applications including pH sensing, corrosion monitoring, environmental sensing and blood gas analysis [2]. In addition, the reference electrode has a pronounced influence on the measurement accuracy of electrochemical bio-sensors [3], bio-impedance probes [4] and biological field-effect transistors (BioFETs) [5], especially for low analyte concentrations. For example, a reference electrode is used to impose a bias voltage on the gate of a BioFET that has been functionalized with a biorecognition probe molecule such as a DNA [6]. The subsequent hybridization of a target complementary DNA strand from the sample solution causes a small change in the transistor characteristics (equivalent to ~ 2 mV change in gate voltage) that is then read out. The accurate sensing hybridization using the BioFET is therefore critically dependent on having a highly stable gate potential imposed through a reference electrode. Several kinds of reference electrodes including the standard hydrogen, calomel and Ag/AgCl electrodes have been used for this purpose, of which the last one is the most versatile. However, macroscale reference electrodes cannot be integrated with miniature BioFETs and associated conditioning and processing electronics. The recent trend towards miniaturization of biosensors has also led to the design and development of miniaturized reference electrodes [7]. However, factors such as potential drift, liquid junction effects and influences of pH and salt concentration of analytes, contribute to loss of stability and short lifetime in these miniaturized reference electrodes [2]. In this paper, we design, fabricate and characterize a microfluidic Ag/AgCl reference electrode that has a stable potential (<0.1mV) for at least 100 hrs.

THEORY AND DESIGN

The Ag/AgCl reference electrode provides a stable potential and is the easiest to miniaturize. Therefore, it has been chosen for this bio-sensing application. The Ag/AgCl electrode consists of a Ag wire partially converted to AgCl, immersed in a standard 1M KCl (internal solution) kept in a glass container. The bottom tip of the container is made of nanoporous glass that is only permeable to hydrogen ions. The equilibrium potential of the Ag/AgCl reference electrode is given by the Nernst equation [1]:

$$E=E^0-(RT/F) \ln (C_{Cl^-}) \qquad (1),$$

where E is the equilibrium potential, E^0 is the standard reduction potential of the electrode against the standard Hydrogen electrode, R is the universal gas constant, T is the temperature in Kelvin, F is Faraday's constant, and C_{Cl^-} is the concentration of chlorine in inner electrolyte. The nanoporous glass membrane prevents the migration of other analytes in the external solution and maintains a stable potential at the electrode by maintaining a stable concentration in various mediums. However, this introduces a liquid junction at the membrane where the internal and the external solutions interact. The potential at the liquid junction depends on various factors, some of which can be static, such as convective flow, and others that can be dynamic, such as electrophoresis and diffusion [8]. The maintenance of this liquid junction potential is critical for the stability of the overall potential of the electrode. One ingenious method that has been employed for macroscale electrodes was to introduce a steady convective flow through the junction. This is known as free-diffusion liquid junction and it minimizes the electrophoretic and diffusional effects and generates a stable and static junction potential [9]. This method has produced stability of ~0.1 mV with minimum effect of pH and external analyte concentration. However, complexity of electrode design, fabrication methods and its operation has prevented miniaturization of this format. In our work described here, we present a microfluidic reference electrode with free-diffusion liquid junction and characterize the effect of various parameters such as the flow, internal solution and external solution on the stability of potential.

EXPERIMENTAL METHODOLOGY

Here, the fabrication procedure for our microfluidic (μF) reference electrode is outlined. SU-8 is patterned on Si using photolithography to form the master mold. Polydimethylsiloxane (PDMS) prepolymer was cast onto it and thermally

(at 65 C for 2 hrs) polymerized in order to fabricate the microchannel (3 cm x500μm×80μm). A 200-μm diameter Ag wire was anodically polarized using a pulsed asymmetric square wave potential (2V, -200mV, 0.5Hz, 15min) in 1 M KCl with a platinum wire as counter electrode. This pulsed deposition technique has been shown to generate nanosheets of AgCl on Ag wire that has higher surface area and improved stability over conventionally deposited AgCl with globular morphology [10]. The Ag/AgCl wire was then inserted into the microchannel reservoir in such a way that only the AgCl layer is exposed. A thin (6μm) polycarbonate (PC) membrane (100 nm pores) was bonded on the PDMS microchannel and it serves as the top wall of the microchannel. The PC membrane was microcontact printed with PDMS prepolymer to define a small (500μm×500μm) area that serves as the interface between the microchannel and the external solution. Finally, a PDMS top layer (~1mm) (with a central opening for the interface region) was bonded using microcontact printing to enclose the microchannel (Figure 1). After attaching interconnections, the microchannel was filled with 1 M KCl as the internal solution. To ensure that the nanopores in the interfacial membrane are filled, the miniaturized electrode was dipped into 1M KCl followed by application of an AC waveform with amplitude of 5V and frequency of 1 Hz, for 10 min across the membrane, or instead, the internal solution was flowed (1M KCl) at 2 μL/ hr for 5 hrs through the membrane.
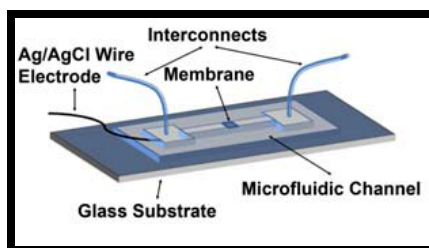

Figure 1: Schematic of the μF reference electrode

RESULTS AND DISCUSSION

Stability of potential and lifetime of the microfluidic reference electrode was characterized against a macroscale commercial Ag/AgCl reference electrode in various modes of operation to determine the optimal configuration for stability of the μF reference electrode. Figure 2(a) shows one configuration, where the microchannel was filled with internal solution (1M KCl) and sealed. Figure 2(b) shows another configuration where a steady flow could be initiated, by means of a syringe pump, either in the microchannel itself or through the nanoporous interface. In all of the experiments, the reference electrode was immersed in 10x Phosphate Buffer Saline (PBS) and the open circuit potential was measured using a semiconductor parameter analyzer (Agilent 4156C). Also, the variance of the potential in a 1 hr averaging window was computed and averaged over 100 hours to compute its stability. The difference between maximum and minimum potential over the entire lifetime was defined here as drift. In addition, the effect of the pH of the external solution (10x PBS) was tested by adding acetic acid and sodium phosphate to decrease and increase pH, respectively. Also, KCl solutions at concentration from 10mM to 3M were used to investigate effect of external chlorine concentration on the potential of the μF reference electrode.
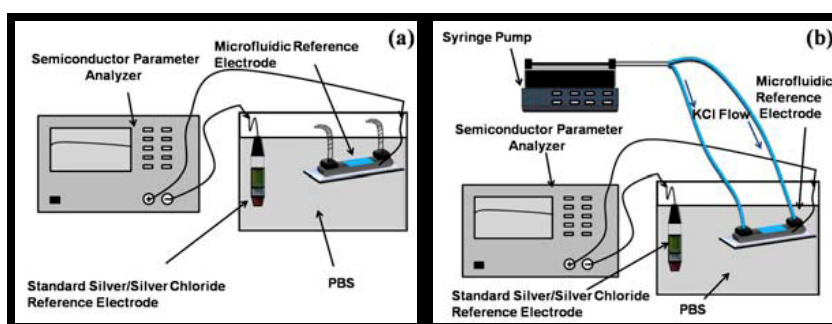

Figure 2: Schematic of stability test set up of μF reference electrode with (a) liquid junction (b) free-diffusion liquid junction

The μF reference electrode (regardless of its configuration) was found to have a longer lifetime (> 100 hrs) when compared to bare Ag/AgCl electrode (63 hours) (Figure 3). Open circuit measurements of bare Silver/Silver Chloride wire showed 2.2 mV drift over 63 hours lifetime with a stability of 34 μV. The μF electrode with a stationary internal solution had a drift of 5 mV over 100 hrs with a stability of 45 μV, as is expected due to the presence of a liquid junction. A flow of 1 M KCl (1 μL/min), through the microchannel, produced a drift and stability of 1.7 mV and 27 μV, respectively over 100 hrs. This result demonstrates the effect of convective flow in minimizing the variation in potential due to diffusion across the membrane. Finally, a convective flow of the internal solution at 2 μL/hr through the membrane results in 1 mV drift and 40μV stability, respectively over 100 hrs. This result demonstrates the importance of minimizing the electrophoretic effect to reduce the variation in junction potential. Figure 4 shows how the bare Ag/AgCl

electrode responds to the variation of chlorine in external solution and is based upon Nernst equation (equation 1). In contrast, the microfabricated reference electrode with stationary internal flow is insensitive to the aforementioned variation. This can be explained by considering the nanoporous membrane as a barrier for external chlorine to diffuse into the internal solution. Similarly, and for the same reason, it does not demonstrate any sensitivity to the pH of external solution, although this couldn't be tested in a basic solution due to dissolution of the membrane at pH higher than 8. Also, the bare Ag/AgCl electrode was tested in solutions with various pH values. Figure 5 indicates that there was a significant fluctuation in the bare electrode potential by changing the pH of the external solution. The pH of the solution affects the solubility of AgCl and therefore affects the bare electrode, resulting in variation of potential. On the other hand, the AgCl in the µF electrode was protected by the inner solution and the membrane and it produces a stable potential.
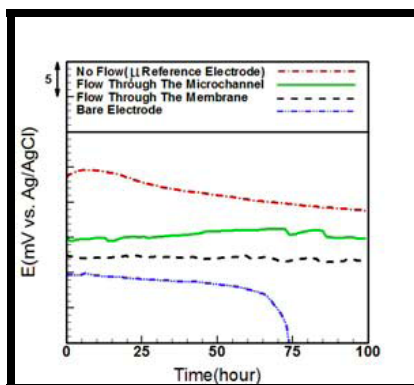

Figure 3: Open circuit potential measurement between reference electrodes and commercial Ag/AgCl in PBS.
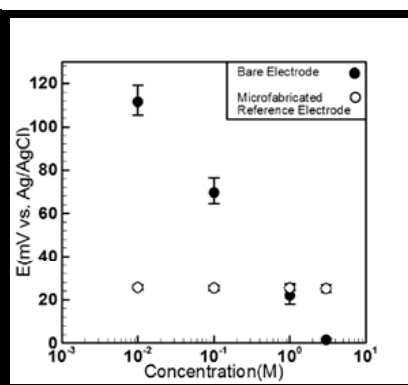
Figure 4: Effect of pH of external solution on µF reference electrode (without flow) and bare electrode stability in PBS as external solution against commercial Ag/AgCl
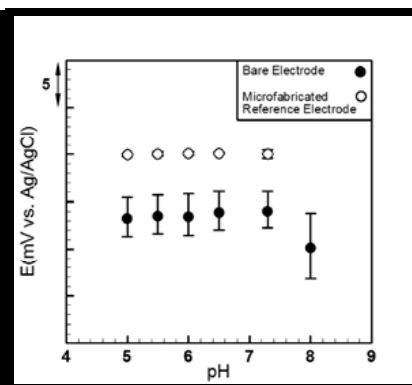
Figure 5: Effect of Chlorine concentration of external solution on µF reference electrode (without flow) and bare electrode stability in KCl as external solution against commercial Ag/AgCl

CONCLUSION

A new microfluidic reference electrode with very high stability (<40 µV) and long lifetime (>100 hours) has been fabricated. It was found that the flow of the internal solution through the membrane can offset effect of liquid junction and therefore make potential more stable, when compared to other configurations. It was also shown that a microfluidic reference electrode with a stationary internal solution is insensitive to the order of magnitude variation of chlorine in the external solution. Finally, the effect of the pH of the external solution on the performance of the reference electrode was studied, and no sensitivity to pH was observed.

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