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Osmotic Sensor for Biomedical Research

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Abstract

Osmotic pressure constitutes a potent new technology suitable for long term tracking of blood glucose. The sensor combines a polymeric nanoporous membrane with a silicon pressure transducer as the sensing element, offering both a potential for miniaturization with reduced power consumption [1]. Albumin was implemented as a model compound for a biochemical assay seeking to identify glucose from other constituents in blood. Its molecular weight (65 kDa) is comparable to the molecular weight of the assay components and the molar concentration change of 1mM generates comparable osmotic pressures to that expected from the assay. The recorded pressure change of 23.4 mBar is lower than the theoretical prediction of 24.7 mBar at 25°C, suggesting that osmotic pressure contribution from larger molecules is less effective than smaller molecular components

Key words: Nanoporous, membrane, osmosis, MEMS, glucose sensor

Introduction

The current thrust in the development of portable biomedical instrumentation is to enable detection at remote locations in contrast to sending samples to large stationary biomedical laboratories of the present [2]. This will allow the patient to obtained data of interest and conduct a self measurement of the state of health related to medical diagnostics, nutrition and environment applications [3]. Those fields are central in biosensor development. According to a review by Connolly [4] the main advantages of implementing biosensors in portable instrumentation is their smaller size and ease of use triggering their applications to grow [2].

A biosensor in its basic form constitutes a device which combines a biological recognition element with a physico-chemical transducer [2, 5]. They are classified into different categories based on: single use, intermittent use and continuous use [6, 7]. Continuous use of the biosensor has been an impetus in medical research with emphasis on the detection of glucose concentration in blood for the patients suffering from diabetes mellitus. Diabetes is a metabolic disorder which entails a reduced or absent blood glucose control in the body [8]. Elevated blood glucose values causes long term damage to the blood vessels resulting in an increased risk of blindness, kidney failure, gangrene, heart failure and stoke [9]. There are two main types of diabetes: Type 1 (juvenile) which is insulin dependent and type 2 (adult onset) which is insulin resistant. Early diagnosis and tight glycaemic control (maintain blood glucose within "normal" limits) will prevent the risk of developing complications later in life [10].

According to the world health organization more than 170 million people have diabetes and those data exhibit tendency to increase [9]. Therefore diagnostic and monitoring of this disease is great importance.

The current standard within glucose sensor technology was developed by Leyland C. Clark, and is based on enzyme sensors [10]. This minimal invasive method requires the sampling of blood by puncturing the finger and placing the droplet of blood on a special strip (sensor) of the measurement apparatus. Diabetics have to make such a glucose test several times a day, and it is both inconvenient and painful, especially for young children and the elderly. Additionally it is impossible to monitor the glucose level in the blood during the night when person is sleeping without having to wake up.

Alternative technologies seeking to replace enzyme sensors for glucose monitoring, are based on optical [11, 12], spectrophotometric [13, 14], spectroscopic [15, 16] and polarimentric [17] methods. Most of them are non-invasive where the measurement system is placed externally outside the body. These indirect measurements of a parameter have resulted in challenges maintaining correct calibration conditions throughout a continues monitoring protocol due to movement of the subject.

Therefore, sensors located *in vivo* (inside the body), represents a promising alternative by enabling the device to perform measurement at the location of the parameter [18]. Such a devise should be minimal invasive by restraining the size, having a simple sensor architecture that reduce power con-

sumption, incorporate inert inorganic material which is not degradable or harmful to the body (biocompatible), permit continuous measurements over extended time frames and incorporate transdermal communication to an external transducer without puncturing the skin. Continuous glucose monitoring represents the best alternative to maintain a healthy glycaemic control and thereby prevent complications from the disease later in life. The quality of life will also be improved both by alleviating stress that manual and incomplete sampling triggers, especially at night.

Osmotic pressure constitutes the diffusion of water down its own concentration gradient [19, 20] through a semipermeable membrane that separates two solutions of different molar concentrations.

This paper presents a pilot study of the implementation of the osmotic sensor technology for the development of a miniaturized blood sugar reader for continues monitoring of blood glucose level. Albumin was used as a model compound for the affinity assay that was tested out in subsequent trails.

Experimental

The osmotic sensor prototype was constructed from a 0.5 mL dialysis cassette (Slide-a-lyzer, Pierce Biotechnology, USA) equipped with a 2 kDa polymer membrane made from regenerated cellulose acting as the nanoporous semipermeable membrane. The membrane permits passage of all molecular components below 2 kDa including glucose (180 Da), salts and water, yet retaining the larger assay components (65 kDa). A 2x2 mm differential MEMS pressure transducer (MS761, Intersema, Switzerland) translated the osmotic pressure into an electric signal recorded on a PC though a DAQ card (National Instruments, USA). The pressure transducer was attached to a silicon carrier with epoxy (Araldite 2020, Vantigo, Switzerland), connected by wire bonding and insulated by Epotek H70 E-2 (Epoxy Technology Inc., USA), fig.1, prior to soldering leads to the carrier.

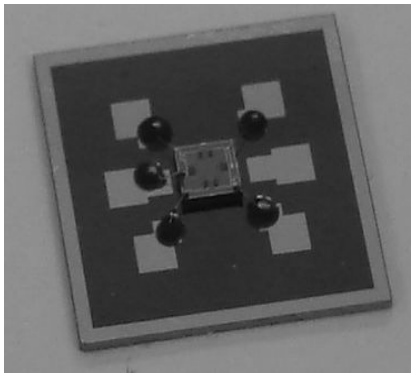

Figure 1: Silicon carrier with attached pressure transducer, after wire bonding and insulation.

A hole was cut in one of the two membranes of the dialysis cassette, enabling the transducer to be attached to the sensor holder (fig.2).

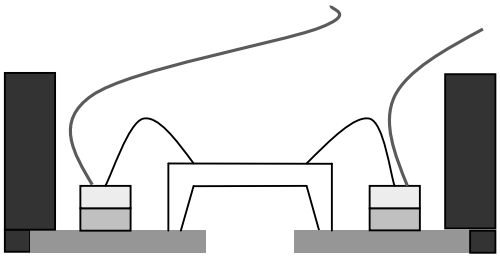

Figure 2: Cross section schematic illustrating the silicon carrier attached to the sensor holder (black).

A reference cavity is formed between the membrane and pressure transducer where the reference solution of a constant osmotic strength is located. The reference cavity is also where the osmotic pressure is generated through interactions between the reference solution and the net particle concentration outside the sensor through the nanoporous membrane. A 30 mm petri dish of polystyrene (VWR, USA) served as the analytical chamber containing the standard solutions (fig.3a) and was attached at the front of the nanoporous membrane with silicon elastomer (Dow Corning 3140, Dow Corning, USA). A hole cut in the bottom of the Petri dish permitted fluid to access the membrane. Likewise a similar dish was attached to the rear face of the sensor/cassette acting as a base for the pressure transducer (fig.3b).

Polydimethylsiloxane, PDMS, (Sylgard 184 Silicone Elastomer, Dow Corning, US) was cast over the transducer to enable electrical insulation of the connections as well as permitting movement and pressure detection by the silicon transducer (fig.3b).

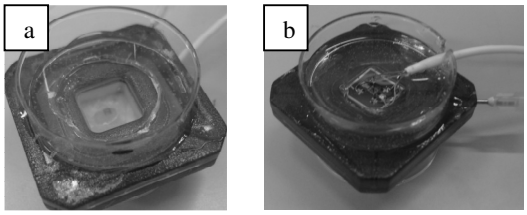

Figure 3: Prototype based on a dialysis cassette. The sensor was encapsulated with PDMS after attachment to the cassette, protecting all electrical connections from the external environment. (a) Top part showing the polymeric membrane, this part of the devise is filled with the standard solution (b) reverse side showing the attached pressure transducer

The reference solution consists of 1 mM albumin, a protein commonly found in blood and

which was used as a model compound investigating sensor function in this system. The protein is available at considerable low cost and offers good solubility in DI water. The molecular weight is approximately the same as the biological assay consisting of the proteins and carbohydrates to be investigated later. The albumin (bovine serum albumin, Cat.No. A9418 Sigma, USA) were dissolved in DI water and degassed at vacuum in a desiccator prior to use. The sensor response was investigated using standard solutions of 0, 0.5 and 1mM albumin. The reference cavity of the sensor was filled (with 0.5 mL of 1 mM albumin) by the aid of a syringe through a port equipped with a self sealing silicone gasket (fig.4). Air was expelled in an iterative manner as liquid was filled using the same syringe and needle.

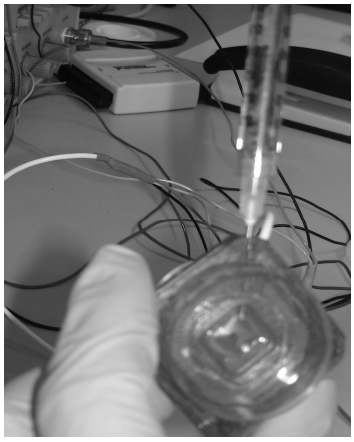

Figure 4: Filling the cassettes with reference solution. As liquid filled the cassettes all air had to be removed in an iterative manner.

The sensor was calibrated with pneumatic pressure from an external source attached to the needle. Temperature calibration was performed by immersing the sensor in a water bath (fig.5). The temperature sensitivity was integrated in a protocol cancelling ambient temperature fluctuations measured by an external temperature probe.

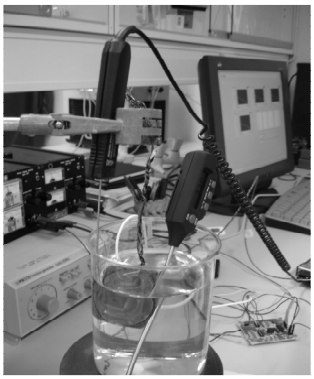

Figure 5: Temperature calibration with the sensor immersed in water.

Standard solutions were aliquoted in 3.5 mL volumes and poured into the analytical chamber. The concentrations of 0 mM (pure water), 0.5 and 1 mM albumin were interchanged by rinsing the chamber with DI water, and repeated 3 times. The membrane was hydrated in DI water for 20 min prior to use. All measurements were performed at room temperature ~ 20°C.

Results and Discussion

An osmotic pressure of - 20.4, -33.6, and – 43.8 mBar was recorded from a transmembrane concentration gradient of 1, 0.5 and 0 mM albumin respectively (table 1).

Table 1: Osmotic pressures using albumin

Transmembrane concentration [mM]	Pressure [Bar]			
	mean	median	Std.	range
0	-0.0438	-0.0438	0.0011	0.0049
0.5	-0.0336	-0.0341	0.0032	0.0141
1	-0.0204	-0.0205	0.0009	0.0038

The negative sign bears significance to the reference point of the sensor. The nature between osmotic pressure and particle concentration suggests that this relationship is linear (fig. 6). Errors could be due to drift in the pressure transducer caused by the silicon carrier of the pressure transducer flexing in response to changing pressures, or water absorption and consequent swelling of the adhesive components used. Any deviations from the 3.5 mL volume used in the standard solutions would trigger an impact through gravitational applied hydrostatic pressure outside the membrane which would trigger a similar response from the pressure transducer b low.

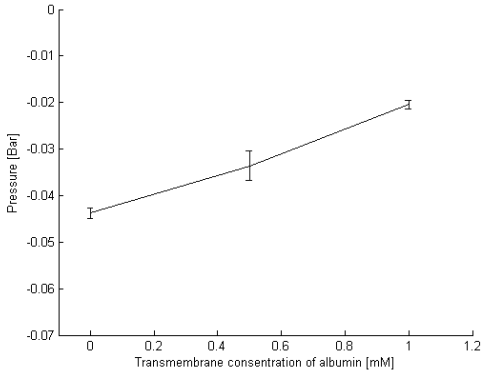

Figure 6: The osmotic pressure [Bar] recorded from a transmembrane concentration gradient of 0, 0.5 and 1 mM albumin

Although the theory states that particle size bears no significant effect on the osmotic pressure

contribution, the result (23.4 mBar measured vs. 24.7 mBar predicted) suggests that larger molecular components like albumin could exhibit a marginally lower osmotic pressure contribution per unit number than smaller molecular components

Conclusion

The results demonstrate the feasibility of applying silicon MEMS devices in recording osmotic pressure from albumin as a model compound. The silicon pressure transducer will be incorporated in a miniaturized sensor for the implementation of assay components identifying glucose *in vivo* from other constituents in blood. The sensor and biochemical assay will be integrated into miniaturizes device measuring 3x7 mm suitable for implantation by injection.

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