Hydrogel-Based Biochemical Sensors

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Abstract

With respect to diabetes management, there is a critical societal need for a sensor that can be used to continuously measure a patient's blood glucose concentration twenty four hours a day on a long-term basis. In this work, thin films of "stimuli-responsive" or "smart" hydrogels were combined with microfabricated piezoresistive pressure transducers to obtain "chemomechanical sensors" that can serve as selective and versatile wireless biomedical sensors. The sensitivity of hydrogels with regard to the concentration of glucose in solutions with physiological pH, ionic strength and temperature was investigated in vitro. The response of the glucose-sensitive hydrogel was studied at different regimes of the glucose concentration change and at different temperatures for two sensor design variants. Sensor response time and accuracy with which a sensor can track gradual changes in glucose was estimated and calibration curve has been obtained.

Keywords: piezoresistive sensor; biochemical sensor; polyelectrolyte hydrogel; glucose-sensitive, pH-sensitive, swelling behaviour, biomedical applications

1. INTRODUCTION

In recent years, numerous attempts have been made to develop a miniature, enzyme-free wireless implantable glucose sensor. Such a device, when implanted in subcutaneous tissue, will continuously measure glucose level, which can be interrogated at will by the patient and also recorded automatically [1-6]. Real-time glucose monitoring can provide maximal information about changing blood glucose levels throughout day and night, including the direction, magnitude, duration, and frequency of such fluctuations. The concept of a feedback loop (sensing-delivery) system goes beyond diabetes monitoring [1-3, 5, 7]. Such ability to deliver an optimal therapeutic dose in response to distinct changes in the body chemistry of each person offers a unique opportunity to deliver personalized medical care and will dramatically change the treatment of other diseases through tailored administration of drugs.
There are still major challenges in achieving clinically accurate glycemic monitoring in connection to closed-loop systems aimed at optimal insulin delivery. It is well reported that fluctuations in oxygen concentration impact glucose oxidase-based sensor performances, both for amperometric enzyme electrodes [7-10] and microfabricated sensors with incorporated glucose-sensitive hydrogel (GSH) containing glucose oxidase enzyme [11]. Hydrogels are crosslinked polymers which swell in solvents to appreciable extent and which are able to a volume phase transition under the influence of external excitations. The amount of solvent uptake depends on the polymer structure and composition, and can be made responsive to environmental factors, such as temperature, electric field, pH-value, ion concentration, salt concentration, analyte concentration in solutions, and solvent composition.
If the GSH is enzyme-free, then the sensor response will be independent of blood oxygen level. In recent years there has been a considerable effort to develop enzyme-free GSHs. The glucose response of these hydrogels is due to incorporated phenylboronic acid (PBA) side chains. In the presence of glucose, these polymers alter their swelling properties, either by ionization or by formation of glucose-mediated reversible crosslinks [1, 4-6].
In this paper, we use metabolically sensitive enzyme-free hydrogels as chemo-mechanical transducers in piezoresistive biochemical sensors. Beside a dip sensor containing the porous membrane, a sensor design based on a direct contact of the hydrogel with the measured solution has been used in order to investigate the direct dynamic response of the hydrogel to the glucose concentration changes in solution. In order to extend the lifetime and efficiency of implanted biosensors, a number of in vitro testing procedures have been performed to evaluate the functionality of glucose sensors.

2. EXPERIMENTAL

2. 1 Sensor design

Piezoresistive chemical sensors operate by monitoring the analyte-induced volume expansion of a thin polymer layer used as chemo-mechanical transducer (Fig. 1). Pressure sensor chips with a flexible thin silicon bending plate and with an integrated piezoresistive Wheatstone bridge at the plate surface have been employed as mechano-electrical transducer for the transformation of the plate deflection *w* into an appropriate electrical output signal V_{out}. For the design of the chemical sensor, commercially available pressure sensor chips (AktivSensor GmbH, Stahnsdorf, Germany) were used.

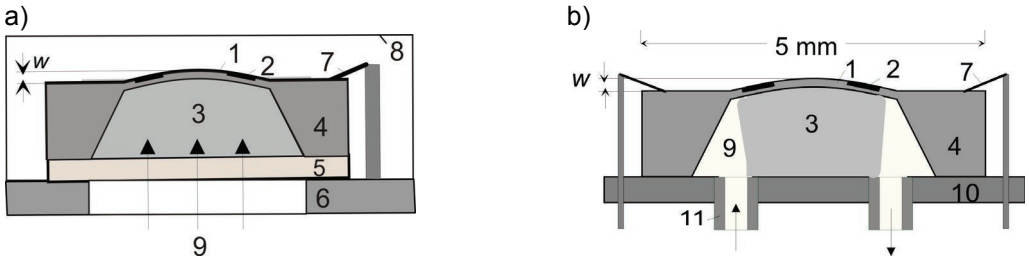

Fig. 1 Design variants of hydrogel-based biochemical sensors: 1 bending plate (3 mm x 3 mm x 0.02 mm); 2 mechano-electrical transducer (piezoresistive bridge); 3 swellable hydrogel; 4 Si chip (5 mm x 5 mm x 0.4 mm); 5 porous membrane; 6 substrate; 7 interconnect; 8 cap; 9 measuring solution; 10 socket; 11 inlet tube

In the piezoresistive biochemical sensors for the measurement of concentrations of chemical species in aqueous solutions, the polymeric hydrogel itself was brought into a cavity at the backside of the silicon chip closed with a porous biocompatible and hydrophilic Anopore^{TM} Al_2O_3 membrane (thickness of 60 µm, pore size of 200 nm) (Fig. 1a). This membrane provides a low protein binding for permanent implants. The solution and analyte molecules diffuse into the chip cavity through the membrane, induce the swelling or shrinking processes of the hydrogel, and lead to the change in the silicon plate deflection. The plate deflection due to the swelling pressure change causes a stress state change inside the plate and therefore a change of the resistivity of the piezoresistors affecting proportionally the output voltage V_{out} of the sensor. An increasing value of V_{out} corresponds to a hydrogel swelling, whereas a decreasing one corresponds to a deswelling.

Only the backside of the chip came in contact with the measuring species, whereas the front side carrying the electronic components was strictly protected from it. Since the sensor chips show excellent stable and dynamic properties with a response time t < 1 s, the long-term stability of the sensor and its response time are solely determined by the stability of the hydrogel characteristics and by the gel swelling/deswelling kinetics, respectively [12-14]. Because the gel response is typically diffusion-driven, the time response of the volume change approximately follows the square of the sample dimension. Scaling to micro-dimensions enhances the time response. Consequently, a reduction of the sample size improves the sensor performance. However, a reduction of the gel thickness is limited by the necessity to obtain a sufficiently high sensor signal and consequently, a sufficient sensitivity.

In order to eliminate the influence of membrane permeability on the sensor response time, a sensor design with the pumped solution was used (Fig.1b). The sensor chip was bonded to a socket with inlet and outlet flow channels. The aqueous solution to be measured has been pumped through the inlet channels into the chip cavity with a flow rate of 0.8 ml/min. The sensor's output voltage was measured during the swelling/deswelling of the hydrogel layer under variable, tightly controlled ambient conditions. The temperature was controlled with a Vaisala HMP 230 humidity and temperature sensor with an uncertainty of ΔT = ± 0.1 *K*. The uncertainty of the temperature setting was ΔT = ± 0.5 *K*.

2. 2 Hydrogel material preparation

A cross-linked glucose-sensitive copolymer of acrylamide and 3-acrylamidophenylboronic acid (AAm/3-APB) was used in the present work.

The monomers used for preparation of the gels were obtained as follows: acrylamide (AAm, Fisher Scientific), *N*,*N*-methylenebisacrylamide (BIS, Sigma-Aldrich), and 3-acrylamidophenylboronic acid (3-APB, Frontier Scientific, Logan, UT). The monomers were used as received. Ammonium peroxydisulfate (APS, Sigma-Aldrich), *N*,*N*,*N´*,*N´*-tetramethylethylenediamine (TEMED, Sigma-Aldrich), and phosphate-buffered saline solution (PBS, Sigma-Aldrich) were also used as received.

A GSH containing AAm/3-APB/BIS at a nominal mole ratio of 80/20/0.25 was prepared by free-radical crosslinking copolymerization as described in [1]. In brief, stock solutions of AAm and BIS were prepared

in distilled water. Appropriate amounts of the two stock solutions were mixed in a vial with TEMED. In order to dissolve 3-APB into the pregel solution, 1M NaOH was added into the vial. The free-radical initiator APS was introduced after purging the vial with N_2 gas for 10 min, after which the pregel solution was rapidly injected into a cavity (thickness 400 µm) between two square plates (polycarbonate and poly(methyl methacrylate)) of surface area 60 cm^2. The total monomer concentration in the pregel solution was 30.2 wt%. After approximately 16 h of reaction at room temperature, the hydrogel slab was removed from the mold and washed for at least two days with deionized water and PBS buffer (pH 7.4, ionic strength 0.15M) before testing. In sugar-free PBS buffer at physiological pH and ionic strength, GSH contains 58 wt% water. The dried hydrogel foils (thickness d_0 = 330 µm) were prepared by evaporation of water at room temperature and then cut into pieces. The pieces of 3 mm x 3 mm were used for the sensor design in Fig. 1a. In the case of the design in Fig.1b, the gel pieces of 1 mm x 1 mm were glued to a socket.

2.3 Solution characterization

D(+)-glucose (Roth) was dissolved in phosphate buffered saline (PBS, pH7.4) solutions of ionic strength I = 0.15 M. The pH value of PBS was adjusted with HCl or with NaOH and was measured using a Knick pH meter with pH/Pt-1000 probe.

3. RESULTS AND DISCUSSION

The AAm/3-AAmPBA gel was investigated as a functionalised polymer coating in piezoresistive biochemical sensors using an appropriate measuring setup. After the sensor preparation, an initial gel swelling in a glucose-free PBS buffer (pH 7.4, ionic strength 0.15 M) was performed at room temperature for 24 h. The value $V_{out,0}$ of the sensor output voltage at steady state of the swollen gel was determined and then used as reference value.

The gel sensitivity with regard to glucose concentration in solution was investigated in vitro. The swelling process of the glucose-sensitive AAm/3-AAmPBA hydrogel due to the change of the glucose concentration c_G in PBS solution was monitored by corresponding change ΔV_{out} in the output voltage of the piezoresistive pressure sensor chip (Fig. 2a). PBA-containing GSH shows volume increase with increasing glucose concentration in PBS solution (pH7.4). The initial rate of the volume increase depends on the step value of the gradual glucose concentration change when started from a glucose-free PBS solution. The initial rate v of the relative increase in the output voltage at the time $0 < t_m < 3$ min has been used in this work for the sensor calibration:

$$v = \frac{\Delta V_{out}}{V_{out,0}} . \qquad (1)$$

Fig. 2b shows the values of the initial rate v in dependence on the glucose concentration c_G in PBS solution (pH7.4) for two sensor design variants.

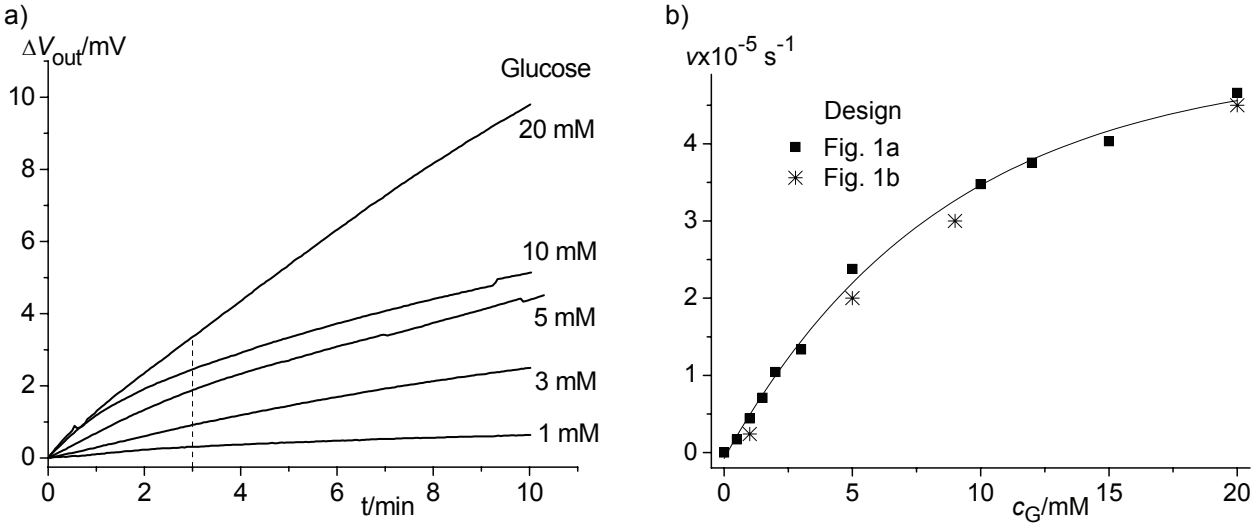

Fig. 2. Time-dependent sensor response for different glucose concentration c_G steps in PBS solutions (pH 7.4, ionic strength 0.15 M) initially starting from c_G = 0 mM (a) and corresponding dependence $v = f(c_G)$ (b).

The pendant groups of phenylboronic acid being present in both an uncharged trigonal form and a charged tetrahedral boronate form in a wide range of pH (from 6.5 to 9 for different substituents) [15, 16].

The sugar-reactive form of boronic acid as well as pendant phenylboronates are anionic. The charged tetrahedral boronate is in a favourable configuration to undergo bidentate condensation with diols such as glucose. Thus, glucose stabilizes the charged form of phenylboronate [6].
The charged form is characterized by a pK value, which in the absence of glucose is given by pK_0 = 8.86 [4]. Binding of glucose is characterized by a dissociation constant of the glucose molecule to PBA, K_G = 9.1 mM. The fraction α of the ionized PBA groups depends on local pH value and on the glucose concentration c_α in the gel according to [3, 4]

$$\alpha = \frac{1 + c_G / K_G}{1 + \lambda \cdot 10^{pK_0 - pH} + c_G / K_G}, \qquad (2)$$

where λ is the Donnan ratio for the mobile cations. Hence, when the environmental glucose concentration increases, the fraction of charged boronic acid groups increases too, thereby increasing the gel hydrophilicity what in its turn leads to the gel swelling.

Copolymers of acrylamide demonstrated the increased gel swelling with increasing temperature (Fig. 3). The gel, initially swollen in PBS solution at room temperature, swells further with increasing temperature. A piezoresistive biochemical sensor offers a perfect possibility for on-line monitoring of the gel behaviour kinetics (Fig. 3a). The temperature characteristic $V_{out} = f(T)$ has been obtained (Fig. 3b) using a very slow natural cooling occurred within 30 hours.

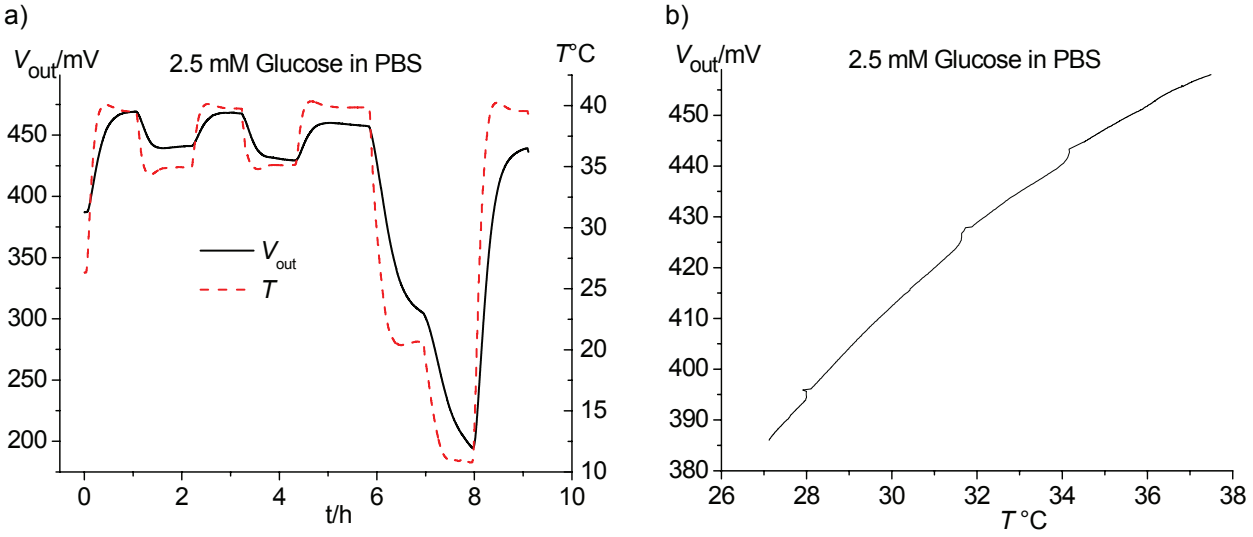

Fig. 3. Time-dependent sensor response due to temperature changes between 10 and 40 °C in a PBS solution (pH 7.4, ionic strength 0.15 M) with c_G = 2.5 mM (a) and temperature characteristic $V_{out} = f(T)$ under slow natural cooling (b).

Since 9 months we have been testing the operational and storage stability of the sensors to assure the sensor will perform sufficiently in vivo. In order to provide the required high signal reproducibility as well as in order to maintain a sufficient long-term stable sensitivity, the sensors have been stored between measurements in a PBS buffer (pH 7.4, ionic strength 0.15 M) at constant c_G = 2.5 mM.

4. CONCLUSIONS

The sensor performance test has been carried out in vitro for glucose sensors based on the glucose-sensitive copolymer of acrylamide and 3-acrylamidophenylboronic acid. Combining a smart hydrogel and a micro fabricated pressure sensor chip in piezoresistive biochemical sensors allows to continuously monitor the glucose-dependent swelling of a hydrogel and hence the glucose concentration in ambient aqueous solutions. The response of the glucose-sensitive hydrogel was studied at step changes of the glucose concentration in solutions with physiological pH and ionic strength. Sensor response time and accuracy with which a sensor can track gradual changes in glucose was estimated.
Not only the measuring principle was adapted to glucose in aqueous solutions but also the implemented sensor has been used as an instrument for on-line monitoring of the gel behaviour kinetics. Additional sensor tests have been carried out for gradual temperature changes. The temperature characteristic of the sensor output voltage has been obtained and will be used for the calibration of the glucose sensors.
The long-term measurements in this work have shown that the life time of piezoresistive biochemical sensors under harsh physiological conditions can be prolonged up to several months.

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