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Effect of Temperature Changes on the Performance of Ionic Strength Biosensors Based on Hydrogels and Pressure Sensors

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***Abstract*— Stimuli responsive hydrogels show a strong ability to change in volume with changes in selected environmental properties. This tendency of these hydrogels to change in volume is captured as pressure-change in confined cavities of pressure sensors. An array of pressure sensors on a single chip may carry hydrogels sensitive to multiple, selected metabolic markers and continuously monitor multiple vital parameters simultaneously. Currently, such sensors are capable of continuously monitoring pH, ionic strength, glucose levels and temperature in the sensor environment. In this paper, we report the effect of temperature changes on the performance of ionic strength sensor. A formulation of hydrogel that renders it sensitive to changes in ionic strength was UV polymerized *in situ* in piezoresistive pressure sensors with different membrane sizes. The sensor sensitivity, response time and stability are investigated as a function of temperature *in vitro*. The effect of temperature on these sensor characteristics is discussed.**

I. Introduction

THEfast growing field of biomaterials and biotechnology fuels a growing demand for the development of new biosensors. For most biosensing applications the sensors should not only be fast and operate continuously but also be highly sensitive and small [1,2].

Stimuli sensitive hydrogels are cross-linked polymeric gels that can absorb large amounts of fluidsupon trigger [3]. Changes in the environment, e.g. temperature [4] or ion concentration change [5], can lead to a change in the hydrogel volume[1,6,7].

Depending on the structure of the hydrogel the biosensors can be used inter alia for the detection of pH change [9-11], ionic strength [12] or glucose concentration change [13]. For this work ionic-strength hydrogels are used to detect changes in salt concentration.

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In order to evaluate the stability and sensitivity of the biosensors a number of *in vitro* tests are necessary [14]. This work focuses on sensor-performance tests at elevated temperatures. The sensitivity, response time and stability of a hydrogel biosensor are examined at few temperatures around 37 °Cand are compared to measurements at room temperature.

II. Materials and Methods

A. Sensor

The sensors used for this work are piezoresistive pressure transducers containing potassium hydroxide (KOH) etched cavities to release 10-15 µm membranes for sensing the changes in the volume of the hydrogel confined in the cavity. Each sensor platform contains 2×2 array of sensorswith membrane sizes of1.5×1.5 mm^2,1.25×1.25 mm^2 and two 1×1 mm^2. The membrane in each sensor contains perforations of 20 µm diameter to enable the flow of the environmental media into the cavities containing the hydrogels. Figure 1 shows the image and an electron micrograph of a sensor.This pressure sensor transduceschanges in the pressure inside the cavity into an electrical output [6].

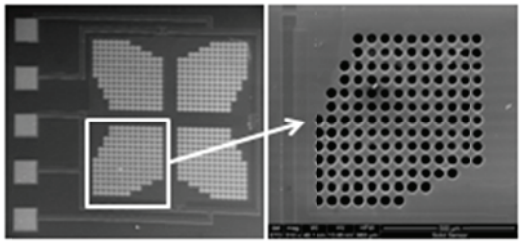

Figure 1: SEM image of a perforated diaphragm sensor. Image shows the pattern of perforations optimized to create maximum strain in the piezoresistors.

The hydrogel is confined in the cavity with the help of a rigid microporous silicon backplate and a mechanical clip. The backplate has perforations with a pore size of 100 µm for the diffusion of media into the cavity. The backplate and the sensor cavity create an isochoric condition for the hydrogel thereby converting its volume change to pressure change on the sensing diaphragm. Four piezoresistors that form a Wheatstone bridge are included in the membrane. The volume changes in the hydrogels cause a deflection in the piezoresistive diaphragm causing a resistance change in the Wheatstone bridge which results in a voltage change from the sensor. The principle of the working of the sensor can be seen in Figure 2.

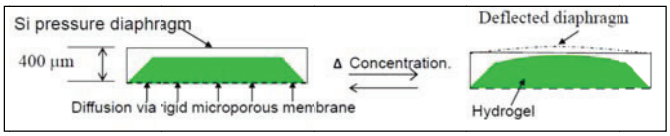

Figure 2: Schematic representation of the deflection of the diaphragm. Adapted from [15]

B. Hydrogel

The hydrogels used for these experiments are sensitive to the changes in ionic strength in the surrounding media. The hydrogel chemistry is composed of the monomers acryl amide (AAM) and dimethyl aminopropylacrylamide (DMAPAA) (Sigma Aldrich) in the molar ratio AAM/DMAPAA: 80/10

The sensing mechanism of the hydrogel is based on the protonation of the tertiary amines of the DMAPAA backbone. A large osmotic swelling pressure can be obtained by increasing the chemical potential of the surrounding water by reducing the environmental ionic strength. This results in a swelling of the hydrogel to compensate for the increase in the osmotic pressure. Hence, the hydrogel swells when moved to a lower concentration solution and deswells/shrinks when moved to a higher concentration solution.

The hydrogels were mixed in the above ratio to form a pregel solution and were photo-polymerized *in situ* in the cavities with the photo-initiator, HHMP/V-pyrol (Sigma Aldrich). The photo-initiator is activated upon excitation at a wavelength of 365 nm to initiate the polymerization reaction in the hydrogel pregel solution. The hydrogel pregel solution was exposed to the UV light (Dymax corporation, Bluewave 75) for 3 seconds at a distance of approximately 1 cm and an intensity of 274 mW/cm^2.

C. Sensor assembly

For the sensor assembly, the sensor array and a PCB connector are mounted on an adhesive tape to provide a stable platform for wirebonding. As a second step they are wire bonded using ultrasonic wedge-bond process with 98% insulated gold-allow wire (Ø = 25µm). The Isonel^{TM} insulation on the wire prevents electrical shorting and also provides a physical barrier between the wires and the surrounding media.

The sutured wires and wirebonds are potted with a medical grade silicone (Nusil technologies, MED 4211) to provide additional encapsulation while increasing the mechanical stability. Finally, a 3 µm layer of Parylene C is chemical vapor deposited on the entire assembly to provide electrical passivation to the sensor array. Parylene C has conformal coating properties and is well accepted as a biocompatible coating and also is insoluble in organic solvents at room temperature [16-18].

The hydrogel is polymerized in the cavities of each sensor and the back plate is put underneath. Back plate and wire bonded sensor are held together via a mechanical clip. The clip allows a reassembly of the sensor after testing as well as a setting of the initial pressure on the hydrogel.

D. Testing methods

For the current experiments with ionic strength sensors, phosphate buffered saline (PBS) maintained at *p*H 7.4 and with ionic concentrations of 100 mM, 125mM, 150mM and 165mM was used. The experiments were conducted with the sensor arrays submerged in PBS in 100 ml polypropylene (PP) bottles that were set into a hot water bath and heated to a set temperature. Tests were initiated once the temperature in the water bath and the PP bottles reached a steady state. The bottle-lids were thermally insulated with a provision to place a digital thermometer for continuous temperature measurements during the testing.

The sensors were conditioned for two days before testing. The sensor arrays were initially placed in 165 mM PBS solution until the signal reached a steady state before shifting it to another PP bottle with a different ionic concentration.

Experiments were conducted at 25, 30, 37 and 45°C. Three cycles were carried out for each temperature and the average of the three cycles was taken. Total of 10 sensors (n=10) were used in these experiments.

III. Results

A. Sensor response

Normalized sensor response to changes in ionic concentrations of all the four microsensors in a sensor array maintained in the water bath at 25ºC is shown in the Figure 3 below.

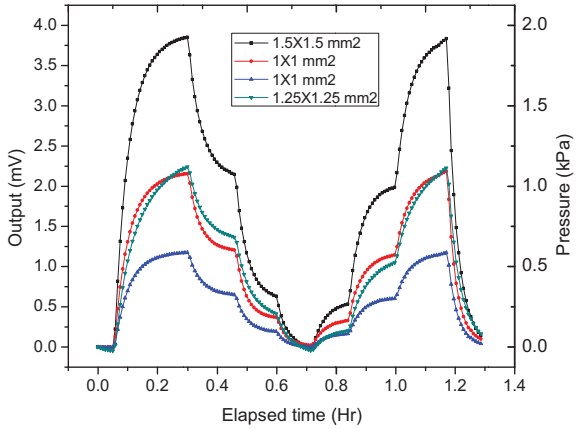

Figure 3: Normalized sensor response over time to changes in ionic concentrations in PBS at 25°C

B. Response time

Response time is the time a sensor needs to reach 63% of its maximum value when the ionic concentration is changed. Response time was calculated using equation 1. This time is of great importance in almost any biosensor [19].

$$y = y_{\circ} + A_1 e^{\left(\frac{x}{t1}\right)} \qquad (1)$$

The time for a big step change (165-100mM PBS) was determined for both swelling and deswelling of the hydrogel. Figure 4 shows the response time calculated for all sensors tested. The standard deviation represents the variations caused by the different sensor sizes.

The swelling of the hydrogel was found to take about 4 minutes at 25°C, 3 minutes for 37°C and 2 minutes for 45°C. The deswelling is approximately one minute faster than the swelling in all the cases.

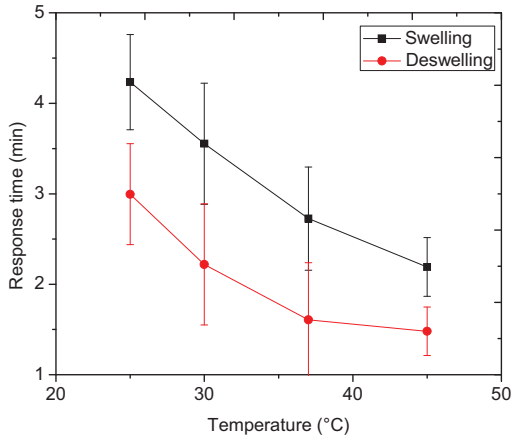

Figure 4: Effect of temperature on the response times of the sensor array for all the diaphragm sizes (n=10)

C. *Sensitivity*

The sensitivity (S) was calculated using equation 2 [20].

$$Sensitivity = \Delta Voltage/\Delta Concentration \quad (2)$$

The range of sensitivities was identified to be different for almost every sensor. It was found that small variations in filling levels of hydrogel in the sensor cavities caused the variations in the sensitivity ranges. For cavities with a smaller amount of hydrogel the diaphragm does not deflect as much and therefore the sensitivity range is smaller. However, the effect of the temperature on the sensitivity of the sensor could still be determined. Figure 5 shows the sensitivity change over temperature for the swelling cycle when the ionic concentration changes from 165 to100mM.

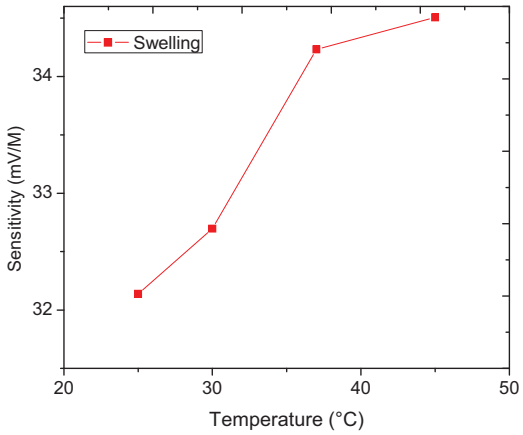

Figure 5: Variations in the sensitivity of the sensor with varying temperature for the swelling step when the concentration changes from 165 to100mM

The sensitivity goes up with temperature by approximately 2 mV/M. The sensor arrays with and without hydrogel was tested in 165 mM PBS while cycling the temperature from 25 to 45°C to investigate the effect of temperature on the hydrogel and the sensor array independently. It was found, that the variation in the output voltage with and without hydrogel in the sensor cavities is negligible compared to the sensor output. This can be seen in figure 6. The output voltage drops about 0.3mV with rising temperature. Small drops in the output voltage can be attributed to unsteady cooling or heating.

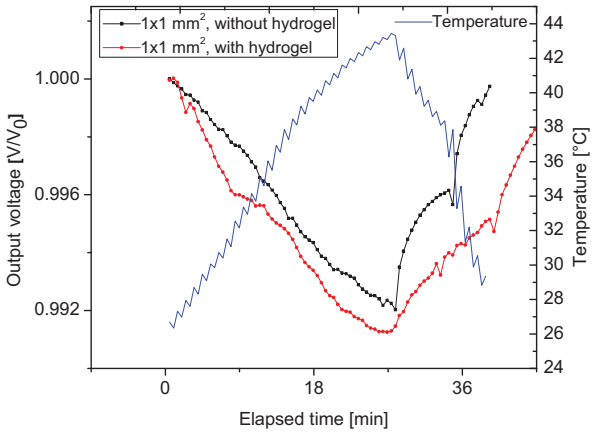

Figure 6: Effect of temperature on the sensor with and without hydrogel at 165mM PBS for a temperature range of 25-45°C with sensor of diaphragm size 1x1mm²

D. *Stability*

The stability of the sensors is the time they function desirably without failure [19]. For each sensor array, three concentration cycles at each temperature were carried out. After 1 week of testing, the sensors were either put into 165mM PBS solution at 37°C or reused with fresh hydrogels. After the completion of all temperature cycles, one concentration cycle was performed every week to ensure proper performance of the sensor. Figure 7 shows the sensor performances over a period of 33 days.

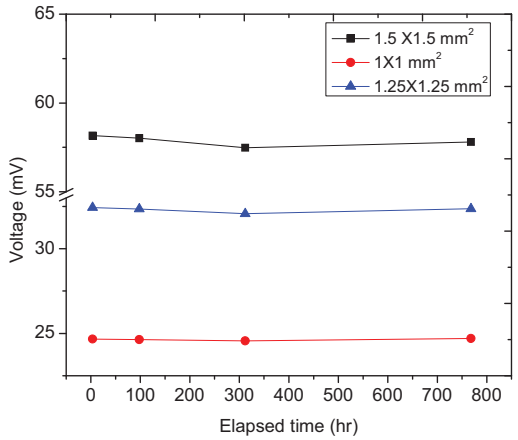

Figure 7: Long-term stability and baseline drift of the three sensor sizes on one sensor array over 30 day at about 36.5.°C

A minor drift in the baseline was detected for all the tested

sensors. The output voltage decreased by an average of about 0.2mV over a period of 33 days.

IV. Discussion

The temperature tests show that the response time for the swelling as well as deswelling cycle is reduced by 50% when shifted from 25°C to 45°C. Due to faster diffusion at high temperatures the solutes can diffuse in and out of the hydrogel at a higher rate which results in a faster volume change and deflection of the sensor diaphragm.

The swelling of the hydrogel was found to be slower than the deswelling by approximately one minute. This could be a result of the additional pressure exerted by the diaphragm on the hydrogel during the deswelling cycle which tends to equilibrate faster than during the swelling cycle. The kinetics of the swelling/ deswelling as well as the thickness of the hydrogel are important factors that are still limiting the response time [11].

It was determined that the effect of temperature on the sensor is a combination of the hydrogel shrinking as well as expansion of the sensor materials. It is believed that due to the expansion of the silicon sensor with temperature the diaphragm is relatively flattened leading to a decreasing output voltage. Simultaneously, the hydrogel shrinks with temperature resulting in a less deflected diaphragm. Small process variations due manual polymerization in the cavities of the sensors also tend to lead to different sensitivity ranges.

The baseline drift is minimal over time but needs to be better controlled or calculated accurately in order to ensure proper functioning of the sensor over time.

V. Conclusion

The performance of the ionic strength sensors was successfully tested in a temperature range of 25-45°C. These sensors were found to have faster response and higher sensitivity at higher temperatures within the tested range. However, the effect of temperature on the sensitivity and the baseline current of the sensor is very small compared to the sensor output. The sensor assembly processes, such as the hydrogel filling level in the cavities, were found to have an effect on the sensor performance besides temperature changes. The effect of temperature change on output voltage in the tested range is smaller compared to sensor output due to analyte concentration change. Therefore the temperature tests show that the sensors can be used at body temperature.

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