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Biochemical piezoresistive sensors based on hydrogels for biotechnology and medical applications

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Abstract. Many conventional analysis techniques achieve a high-detection sensitivity; however, they are equipment or time expensive due to a multi-step procedure. Sensor concepts, introduced in this work, using piezoresistive pressure sensor chips with integrated analyte-sensitive hydrogels enable inexpensive and robust biochemical sensors, which are miniaturized and in-line capable. For these sensor setups, it is important to optimize current established analyte-sensitive, reversible and biocompatible hydrogels for pH and glucose monitoring of chemical and biochemical processes. Therefore, low-viscous monomer mixtures based on hydroxypropyl methacrylate (HPMA), 2-(dimethylamino)ethyl methacrylate (DMAEMA), tetraethylene glycol dimethacrylate (TEGDMA) and ethylene glycol (EG) were prepared in molar ratios of 70/30/01/20, 60/40/01/20 and 60/40/02/20, respectively. Redox-polymerization of these pre-gel solutions were realized with N,N,N′,N′-tetramethylethylenediamine and ammonium persulfate. The reversible pH-sensitive swelling behavior of hydrogels with compositions were compared. By using the photoinitiator 2-hydroxy-4′-(2-hydroxyethoxy)-2-methylpropiophenone, the free radical photopolymerization could be implemented leading to an increase of the swelling degree (SG). Glucose-sensitive hydrogels were prepared via immobilization of glucose oxidase in HPMA–DMAEMA–TEGDMA–EG hydrogel discs. These showed increasing swelling degrees with higher glucose concentrations in aqueous media and a reversible swelling behavior. The synthesized hydrogels were integrated in different piezoresistive sensors of different designs. The pH-depending course of the output voltage of a dip sensor with photopolymerized 60/40/02/20 hydrogel was studied in detail. Besides the usage of a dip sensor, two implantable, parylene C-coated setups are presented. The implantable sensor with long isolated gold bond wires was proved to be functional even after storage in aqueous media for several days.

1 Introduction

Hydrogel-based piezoresistive dip sensors, which can be used in-line or online for control and monitoring of chemical and biochemical processes and for a fast detection of small amounts of solutes, are of great interest for process monitoring in drinking, ground- and sewage water technology, in electroplating as well as in biotechnology, food and pharmaceutical industry. In all these applications, the integration of structured, analyte-sensitive hydrogels with incorporated biomolecules into piezoresistive sensor chips is a challenge. The utilization of such sensors for medical applications is even more challenging due to requirements with respect to implant possibility and biocompatibility.

This work focuses on piezoresistive biochemical sensors that use pH-sensitive, biocompatible, three-dimensional hydrogels with incorporated glucose oxidase (GluOx). They are aimed at the detection of small glucose concentration changes. In the presence of oxygen and water, glucose oxidase catalyzes the enzymatic reaction of glucose into gluconic acid and hydrogen peroxide (Eq. 1).

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{GluOx}} \text{Gluconic acid} + H_2O_2 \qquad (1)$$

In the last 30 to 40 years several efforts have been made to utilize this catalytic reaction in biosensor detecting and measuring glucose (Gough et al., 1982, 1985; Lucisano and Gough, 1988; Wilkins, 1989). The amperometric method, where an electrode produces a current proportional to the

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diffusional flux of hydrogen peroxide or oxygen to the electrode surface, is probably the technique used most often for glucose biosensors. On the other hand, the enzymatic reaction causes pH changes due to the production of gluconic acid. In particular, an increase of the glucose concentration causes a lowering in pH value (Jung et al., 2000). Hence, a GluOx-loaded pH-sensitive hydrogel changes its swelling state dependent on the surrounding glucose concentration. As shown in Ishihara et al. (1984) large swelling changes are possible; they used gels based on copolymers of hydroxypropyl methacrylate (HPMA) and N,N-diethyl-aminoethyl methacrylate (DEAMA) containing GluOx. Changes in glucose concentrations resulted in changes of the pH value within the hydrogel due to the GluOx-catalyzed production of gluconic acid. The gluconic acid protonated the tertiary amine groups of the DEAMA in the gel and produced a charged hydrogel network. Electrostatic repulsive forces between the amino groups increased swelling of the hydrogel. The corresponding swelling degree (SG) of the hydrogel can be detected as a mass change by means of the weighing of the free swollen hydrogel samples, or as a volume change by using a microscope and a charge-coupled device (CCD) camera.

Another possibility is to monitor the hydrogel swelling using a pressure transducer. Herber et al. (2004) studied the behavior of stimuli-sensitive 2-(dimethylamino)ethyl methacrylate-co-hydroxyethyl methacrylate hydrogels under isochoric conditions by enclosing the hydrogel between a micropressure sensor and a porous cover. Water and external stimuli could diffuse through the pores, interact with the hydrogel and provoke swelling, which resulted in pressure generation measured by the pressure sensor. Han et al. (1999) discussed a capacitive pressure transducer for measuring the osmotic swelling pressure of stimuli-sensitive hydrogels. Here a flexible diaphragm is in mechanical contact with the hydrogel and deflects in response to changes in the pressure of the hydrogel. Thereby, the size of an air gap between the diaphragm and an electrode changes resulting in changing values of the capacitance, which were detected remotely using a diode quad bridge circuit. In further publications (Han et al., 2002; Lin et al., 2009, 2010; Orthner et al., 2010a, b; Schulz et al., 2010; Avula et al., 2011; Bates, 2013) pH-sensitive hydrogels made of free radical cross-linked copolymers of HPMA, 2-(dimethylamino)ethyl methacrylate (DMAEMA) and tetraethylene glycol dimethacrylate (TEGDMA) in piezoresistive pressure transducers were integrated. In this way, pH sensors and – by GluOx immobilization within the hydrogel – glucose-sensing biosensors were built.

Within this work redox polymerized and photopolymerized pH-sensitive hydrogels of various compositions were integrated into piezoresistive pressure sensor chips. Hydrogels confined in the chip cavity were directly connected to the thin bending plate of the piezoresistive pressure sensor chip, which will be deflected in case of hydrogel swelling. This plate deformation causes a mechanical stress state inside the piezoresistors, resulting in a resistivity change of the bridge circuit and proportionally effects the electrical output voltage V_{out} (Gerlach and Arndt, 2009; Gerlach and Dötzel, 2008). The hydrogel acts as a chemo-mechanical transducer exerting a stimuli-dependent force to the flexure plate of the piezoresistive pressure sensor chip, which forms a mechano-electrical transducer (Guenther et al., 2009, 2010; Schulz et al., 2010).

This paper presents our first approaches and strategies for the improvement of pH-sensitive hydrogels based on HPMA, DMAEMA, TEGDMA and ethylene glycol (EG), and furthermore a possible glucose oxidase immobilization technique for the production of glucose-sensitive hydrogels. Both redox- and photopolymerized hydrogels of various compositions are examined and compared in terms of their pH and glucose swellability using free swelling studies. Through integration of the polymerized hydrogels into the piezoresistive pressure sensor chips, the suitability as hydrogel-based sensor concepts are assessed. Sensor setups using piezoresistive pressure sensor chips with an integrated analyte-sensitive hydrogel layer enable inexpensive and robust biochemical sensors, which are miniaturized and online capable (Guenther and Gerlach, 2009). In the course of these characteristics, hydrogel-based piezoresistive sensors are also suitable for medical applications, such as in situ measurements of the blood sugar level or other physiological blood values in humans. Subsequently, two partly implantable and biocompatible sensor setups are presented in this work.

2 Materials and methods

2.1 Materials

The following monomers were purchased from Sigma Aldrich and used as received:

- HPMA
- DMAEMA
- TEGDMA.

Furthermore,

- ethylene glycol EG
- N,N,N′,N′-tetramethylethylenediamine (TEMED)
- ammonium persulfate (APS)
- 2-hydroxy-4′-(2-hydroxyethoxy)-2-methylpropiophenone (HEMP)
- β-D-glucose oxidase
- tablets of phosphate-buffered saline (PBS) solution

from Sigma Aldrich were used. The PBS tablets were dissolved in deionized water and the resulting buffer solutions were adjusted with NaOH, HCl and NaCl to an ionic strength value of 0.15 M and selected pH values.

2.2 Standard preparation procedure of pH-sensitive hydrogels

The monomer DMAEMA contains pH-sensitive tertiary amines. HPMA monomers prove for the adjustment of the volume phase transition of the hydrogel to the physiological pH range (Jung et al., 2000). TEGDMA acts as a cross-linker, whereas EG is the solvent. Monomer mixtures from HPMA, DMAEMA, TEGDMA and EG were produced in molar ratios of 70/30/01/20 and 60/40/01/20. The pre-gel solutions were stirred for 30 min under a continuous nitrogen purge.

With the addition of 0.03 vol% TEMED and 2.7 vol % APS solutions into the pre-gel solution, the cross-linking took place via redox polymerization. After stirring for 5 min during a continuous nitrogen purge, the mixtures were transferred into a PTFE (polytetrafluoroethylene) mold with 30 mm × 3 mm × 3 mm hollows. To ensure a uniform hydrogel shaping and an entire polymerization, the fully filled multi-piece mold was closed with a PTFE cover, fixed with screws and stored for 24 h at 4 °C. The demolded beam-like hydrogels were cut into 0.3–0.5 mm thin square (3 mm × 3 mm) disks with a scalpel.

The usage of the photoinitiator 2-hydroxy-4′-(2-hydroxyethoxy)-2-methylpropio-phenone enabled the cross-linking via free radical photopolymerization. The HPMA–DMAEMA–TEGDMA–EG–HEMP pre-gel solutions were stirred with a light exclusion and continuous nitrogen purge for 30 min. The photopolymerization occurred in square molds (5 mm × 5 mm) with a depth of 0.5 mm fully filled with pre-gel solution over 60 s while exposed to ultraviolet light (wavelength ca. 365 nm).

All gel samples were washed for 3 days in deionized water, 1 day in ethanol and for 2 days in a PBS solution (pH 7.4).

2.3 Preparation of glucose-sensitive hydrogels

The washed hydrogel discs (molar ratio 70/30/01/20) were stored in a 1000 mL^{-1} aqueous solution of yellow GluOx powder at 4 °C for 3 days in order to immobilize the glucose oxidase. In this way, the enzyme molecules diffused into the pH-sensitive hydrogel network and dyed the previously transparent gel yellow. Subsequently, the enzyme-loaded gel samples were washed for 3 days in a PBS solution (pH 7.4). The yellowish color remained.

The prepared hydrogels are listed in Table 1.

2.4 pH swelling tests

The hydrogel discs were alternately put into PBS buffer solutions with pH 4 and pH 8 at room temperature for ca. 30 min.

Table 1. Compositions and polymerization methods of hydrogels prepared in this work for free swelling studies.

Hydrogel sample	HPMA–DMAEMA–TEGDMA/–EG (mol %)	Polymerization method	Enzyme
Hydrogel no. 1	70/30/01/20	Redox	–
Hydrogel no. 2	60/40/01/20	Redox	–
Hydrogel no. 3	70/30/01/20	UV	–
Hydrogel no. 4	70/30/01/20	Redox	Glucose oxidase

Afterwards, the discs were taken out of the current pH test buffer, the surface residual fluids were dabbed with a laboratory cloth and the gel pieces were weighed. The swelling degree of the individual gel samples was determined as $SG = m/m_0$, where m is the mass of the hydrogel swollen in pH buffer solution and m_0 is the mass of the hydrogel after storage in deionized water for 3 days.

2.5 Glucose-swelling tests

The gel sensitivity with regard to the glucose concentration in solution was investigated within a physiological range from 0 to 20 mM of glucose. At the beginning of the test series, the glucose oxidase-loaded gel discs were left for 4 h and thereafter for 2 h in the glucose-containing PBS solutions. The weighing procedure was similar to the pH swelling tests.

2.6 Sensor setups

The following sensor setups are based on piezoresistive pressure sensor chips (C41-Series, Epcos, Munich, Germany; size 5 mm × 5 mm × 0.4 mm).

For dip sensors, custom-made circuit boards (Beta Layout, Aarbergen, Germany) were used as substrates (Fig. 1). The cavity of the pressure chip was filled with a washed and conditioned piece of hydrogel and closed with a porous Al_2O_3 membrane (0.2 µm Anopore™, Structure Probe, SPI Supplies, West Chester, Pennsylvania, USA). This stack was positioned above an opening in the circuit board and fixed with glue. The sensor chip and the circuit board were electrically interconnected via bond wires. To protect the setup from aqueous media the electrical components were covered with a silicone cap. The analyte molecules of the aqueous test media diffuse through the opening and through the Al_2O_3 membrane into the chip cavity containing the hydrogel and generate a swelling and, hence, a volume change of the hydrogel. This results in a change of the deflection w of the bending plate. The piezoresistive bridge transforms the change in a mechanical stress within the bending plate and, finally, in an electrical V_{out}. The V_{out} is read out via bond and solder contacts.

Owning to its inexpensive basic components, robustness and susceptibility to miniaturization, it is conceivable to use

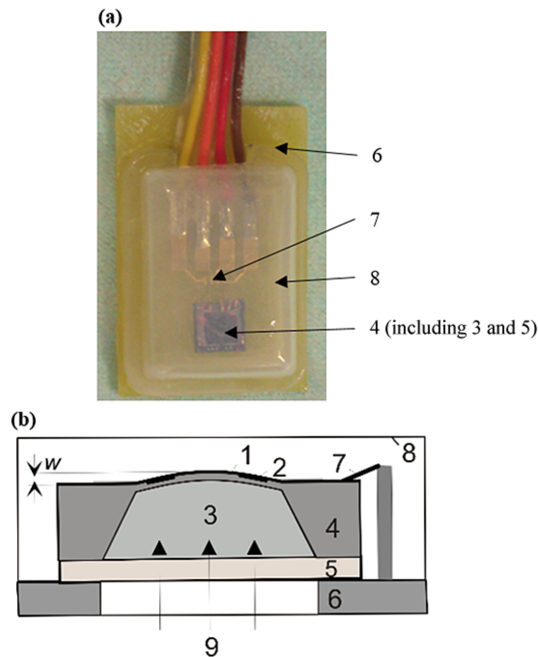

Figure 1. Dip sensor **(a)** and cross section of the sensor element **(b)** (Schmidt et al., 2014): 1 – bending plate, 2 – mechano-electrical transducer (piezoresistive bridge), 3 – cavity with hydrogel, 4 – Si chip, 5 – porous membrane, 6 – substrate with opening, 7 – interconnect, 8 – cap, 9 – analysis solution.

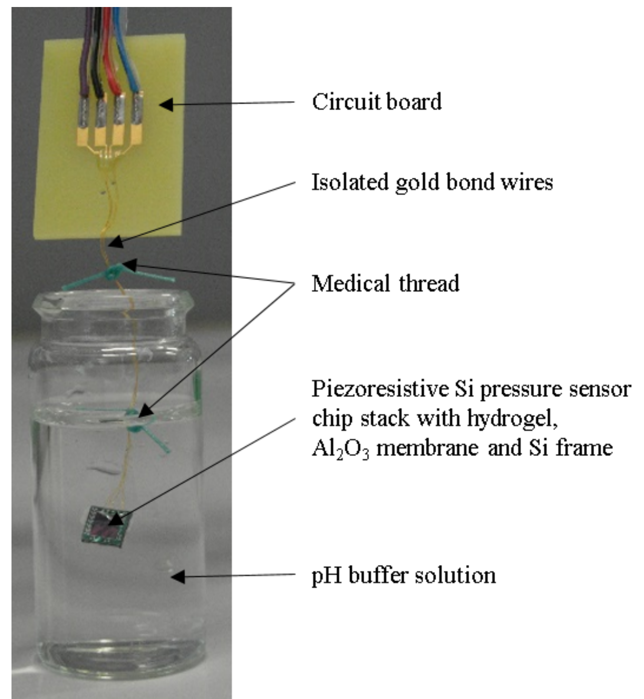

Figure 2. Praylene C-coated implantable sensor with isolated gold bond wires in pH buffer solution.

the presented concept of the hydrogel-based piezoresistive sensor for medical applications, such as in situ monitoring of the blood sugar level or other physiological blood levels in humans. Therefore, the dip sensor has to be converted into an implantable and biocompatible sensor concept. In the following, two developed implantable hydrogel-based piezoresistive sensor setups are described.

The sensor assembly pictured in Fig. 2 contains the pressure sensor chip with a hydrogel in its cavity, the porous Al_2O_3 membrane and a silicon frame instead of a substrate. Long (ca. 4 cm) isolated gold bond wires (X-Wire™ Technologie, Microbonds Inc., Markham, Ontario, Canada) interconnect the sensor chip and the circuit board. To make the bond contacts more reliable, a medical grade epoxy (Loctite M-31CL, Brammer GmbH, Dresden, Germany) was applied onto the bond pads and bonds on both the pressure chip and the circuit board. For curing the epoxy, the assembled sensor was then placed in an oven at 60 °C for 60 min. Afterwards, the bond wires were sewed with a medical thread at various locations along the length of the wire to apply additional mechanical strength to the wires. For a better stabilization, medical grade silicone (MED-4211, NuSil Technology Europe, Mougins, France) was applied dropwise onto each knot and spread onto the wires. The sensor was placed in the oven at 60 °C for 3 h for the silicone curing. Subsequently, the sensor assembly was coated with a ca. 5 µm thick parylene C layer in a CVD (chemical vapor deposition) process (Microelectronic Packaging, Dresden, Germany). The encapsulation with parylene C protects the electrical components from aqueous media and ensures the biocompatibility of the sensor.

Figure 3 shows the test arrangement of an implantable and flexible sensor. Biocompatible polyimide foils (Kapton HN, thickness 50 µm, colorprint tech-films, Frankenthal, Germany) were used as substrates. The individually designed conductor line structures were produced in a gold vapor deposition process using ceramic masks. The pressure sensor chip was connected to the conductor lines by means of flip chip bonding. In order to provide a free deflection of the bending plate, a 3 mm × 3 mm window was cut out in the Kapton foil. Afterwards, a piece of hydrogel was put into the chip cavity and the cavity was closed with an Al_2O_3 membrane. An adhesive Kapton foil was put on the back side of this stack to fix the membrane and protect the gold conductor lines from fluids.

It is worth noting that the pieces of hydrogels were washed before being placed into the sensor cavity and were conditioned by three swelling and deswelling cycles.

2.7 Biocompatibility of Kapton®HN foil

The biocompatibility of the Kapton foil was studied using cell experiments. Human fibroblasts were cultivated in cell culture flasks. We used the CellTiter-Blue® assay (Promega, Madison, Wisconsin, USA) to determine the cell viability.

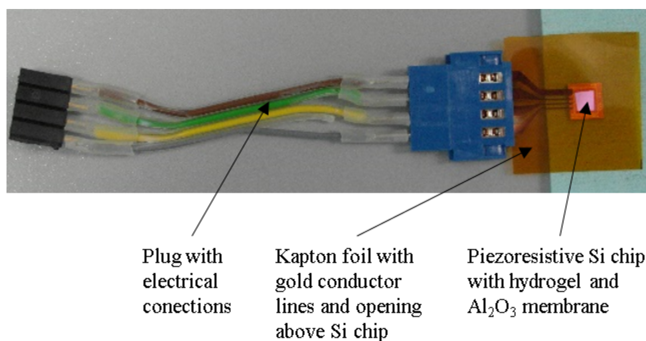

Figure 3. Test arrangement of a flexible, implantable sensor with plug.

For these experiments, cells were cultivated on the Kapton samples in 48-well plates. The foil samples with a 10 mm diameter were sterilized for 5 min in 70 % ethanol, rinsed with double distilled water and dried.

Afterwards, the foil samples were put in the sterile plate. The experiments were conducted with 600 µL cell suspension. The cell concentration amounted to 4×10^4 cells mL^{-1} at the beginning. Then the incubation followed for 72 h at 37 °C, 5 % CO_2 and 80 % relative humidity.

For the evaluation, the excess medium was aspirated after 72 h and a fresh medium with a CellTiter-Blue® assay reagent was added to the adherent cell culture. After 4 h the conversion from resazurin to resorufin was determined by means of fluorescence intensity measurements ($560_{Ex}/590_{Em}$) using a MTP reader (microplate reader; Tecan, Männedorf, Switzerland). The quantity of sample analogies per test were four, and three independent tests were carried out ($n = 3$).

3 Results

Free swelling behavior of the investigated hydrogel discs is depicted in Figs. 4, 5 and 6. Each measuring point of the graphs shown correspond to the arithmetic average of three samples.

3.1 pH swelling tests

Figure 4 compares the swelling degrees of the pH-sensitive, redox polymerized hydrogels nos. 1 and 2 (see Table 1) at cycling between pH 4 and pH 8.

The graphs in Fig. 5 depict the dependence of the swelling behavior on the polymerization method. For that reason, the swelling degrees of the redox polymerized hydrogel no. 1 and the photopolymerized hydrogel no. 3 are compared.

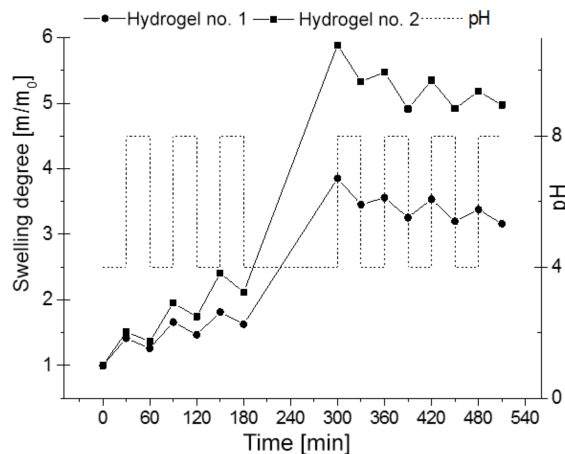

Figure 4. pH-sensitive gel swelling behavior of the redox polymerized hydrogels no. 1 (30 mol % DMAEMA) and no. 2 (40 mol % DMAEMA) (Table 1).

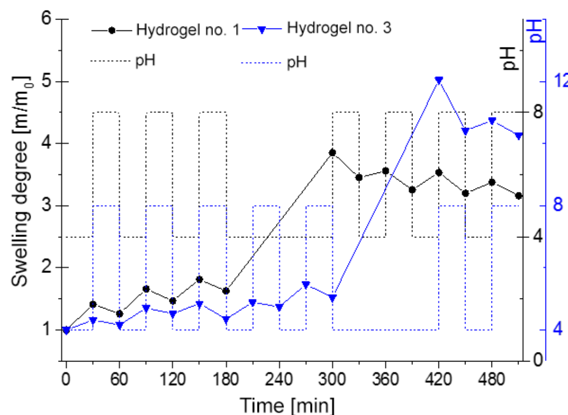

Figure 5. Influence of the polymerization method on the pH-sensitive gel swelling behavior of hydrogels no. 1 (redox polymerized) and no. 3 (photopolymerized) (Table 1).

3.2 Glucose swelling tests

Figure 6 shows the swelling behavior of glucose oxidase-loaded hydrogel discs (hydrogel no. 4; see Table 1) cycling between 0 and 20 mM glucose concentration $c_{Glucose}$ in PBS buffer solutions (pH 7.4) and at $c_{Glucose} = 2$ mM.

3.3 Sensor setups

Figure 7 shows the V_{out} of a dip sensor based on the photopolymerized HPMA–DMAEMA–TEGDMA–EG (molar ratio 60/40/02/20) hydrogel during cycling between pH 4 and pH 8 in PBS solutions. The dip sensor was manufactured using a 300 µm thick square hydrogel disc (3 mm × 3 mm).

Figure 8 shows the course of the V_{out} of an implantable sensor corresponding to Fig. 3 after a pH jump from pH 7 to pH 11. Here, a pH-sensitive poly(vinyl alcohol)/poly(acrylic

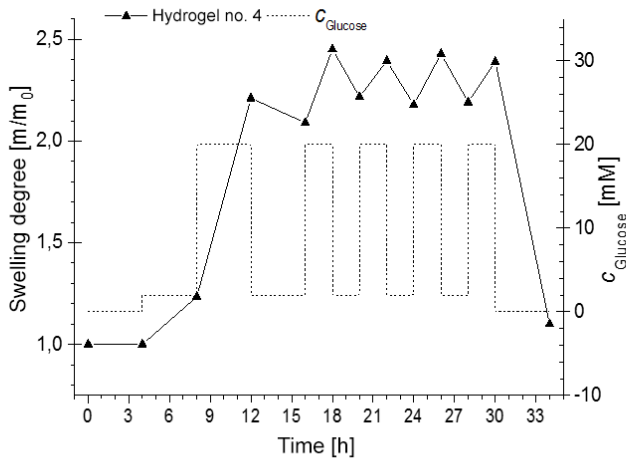

Figure 6. Glucose-sensitive swelling behavior of the GluOx-loaded hydrogel no. 4 in PBS solutions with different glucose concentrations (0, 2 and 20 mM). The PBS-glucose solutions were adjusted to the physiological pH value of 7.4.

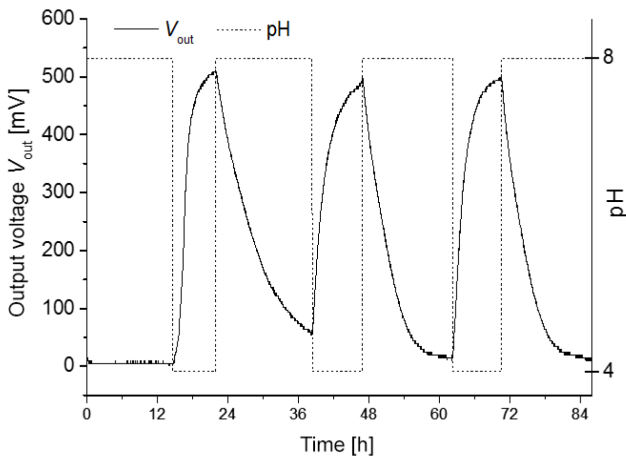

Figure 7. Output voltage (V_{out}) of a dip sensor based on a photopolymerized HPMA–DMAEMA–TEGDMA–EG (60/40/02/20) hydrogel at alternating pH values.

acid) (PVA/PAA) hydrogel was used. The sensor was stored in deionized water for 7 days before measuring V_{out}.

3.4 Biocompatibility of Kapton®HN foil

Cell tests using the CellTiter-Blue® assay were carried out for the Kapton HN foils in order to verify their biocompatibility for their possible usage as substrates for implantable and flexible sensors. Figure 9 depicts the percentaged cell viability of human fibroblasts on the Kapton foils and polyethylene terephthalate (PET) foils.

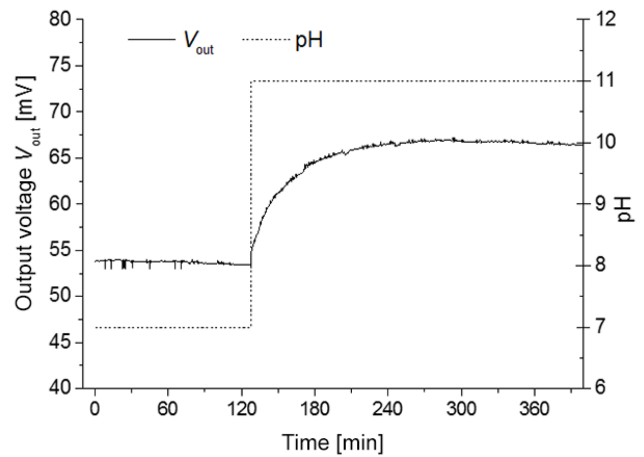

Figure 8. Output voltage (V_{out}) of an implantable sensor (see Fig. 3) with PVA/PAA hydrogel during pH jumping from pH 7 to pH 11 after storage in deionized water for 7 days.

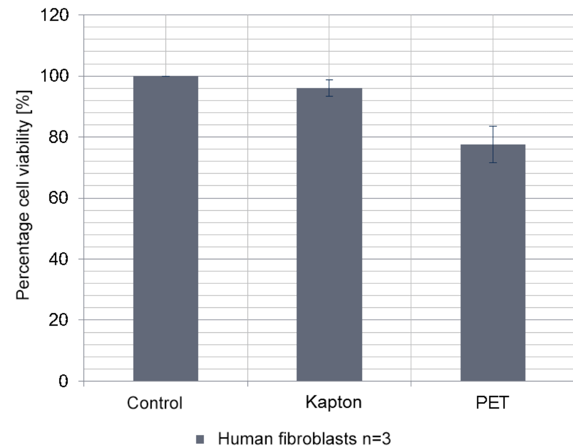

Figure 9. Cell viability tests with human fibroblasts on Kapton and PET foils, respectively.

4 Discussion

4.1 pH swelling tests

The pH-active tertiary amine side group of the DMAEMA are protonated in an acidic environment. This results in a mass increase or swelling of the polymerized HPMA–DMAEMA–TEGDMA–EG hydrogels in a pH 4 PBS buffer solution. In pH 8, the tertiary amine groups are deprotonated and the hydrogels deswell (Figs. 4, 5). As illustrated in Fig. 4, a measurable pH-sensitive change in swelling degree was observed within 30 min. The hydrogel pieces swelled further with increasing exposure time. The swelling degree of hydrogel no. 1 increased from 1.8 to 3.9 in a PBS pH 4 solution. By increasing the amount of DMAEMA from 30 mol % (hydrogel no. 1) to 40 mol % (hydrogel no. 2) the swelling effect was enlarged (Fig. 4).

Using the ultraviolet photopolymerization with a photoinitiator 2-hydroxy-4′-(2-hydroxyethoxy)-2-methylpropiophenone, the swelling degree of the gel with a 70/30/01/20 composition was increased after five conditioning cycles (Fig. 5). In a PBS pH 4 solution, the maximal values of the swelling degree were 3.9 for the redox polymerized hydrogel nos. 1 and 5 for the photopolymerized hydrogel no. 3. For redox polymerized as well as for UV-polymerized hydrogels the swelling degree was influenced by the exposure time in an analysis medium. Due to the longer storage in a PBS pH 4 solution, the swelling degree increased from 1.7 to 5 (Fig. 5; hydrogel no. 3).

4.2 Glucose swelling tests

Even after a 3-day washing process, the color of the glucose oxidase-loaded gel discs remained yellowish. This was expected as an indicator for a successful immobilization of GluOx inside the hydrogel pieces. The glucose swelling tests confirmed the glucose-depending mass change of the hydrogel discs (Fig. 6). The swelling degree increased with increasing glucose concentration (20 mM) in a PBS pH 7.4 solution and decreased at a lower glucose concentration (2 mM). After an initial storage time in glucose-containing PBS test solutions for 4 h, the exposure time was reduced to 2 h. Despite the shorter swelling time, the swelling degree increased from the initially value of 2.2 to 2.45 after only 2 h in a PBS solution with $c_{\mathrm{Glucose}} = 20$ mM. During the following alternating swelling–deswelling cycles, the enzyme-loaded hydrogel discs reached the stable values of the swelling degree 2.4 in 20 mM and 2.2 in 2 mM. The finally measured swelling degree without glucose of 1.1 after 4 h of storage in a PBS pH 7.4 solution showed that the gels deswelled almost to their initial states.

4.3 Sensor principle

Figure 7 proves that pH-sensitive piezoresistive dip sensors with photopolymerized HPMA–DMAEMA–TEGDMA–EG (60/40/02/20) hydrogel show both a sufficient sensitivity and a promising reproducibility. It corresponds well to the results from the free swelling tests (Figs. 4, 5). The curve indicates different response times for swelling and deswelling. In pH 4, swelling is much faster than deswelling in pH 8. This and the long-time response of a few hours for both processes are assumed due to the relatively large pH jump and the complex interaction between the hydrogel and the PBS buffer system. The rate of swelling depends on three factors, which in turn are a function of pH: (1) required change of the ionization state of the charged hydrogel groups to reach a new equilibrium, (2) water flux into the hydrogel and (3) the capability of the buffer to transport protons. For a larger pH-change, a corresponding change of the ionizations state inside the hydrogel is required and more protons have to be transported through the hydrogel to reach the new swelling equilibrium. The ability of the buffer to transport protons is, among others, affected by its salt composition, pH value and, therefore, the ionization state (Schulz et al., 2010). These complex interactions in combination with the hydrogel layer thickness determine the swelling kinetics of the hydrogel. For future experiments the response time should be decreased by thinner hydrogel discs and the usage of particle-based hydrogel samples to accelerate diffusion.

Figure 8 provides evidence of the functionality of an implantable sensor after storage for 7 days in deionized water. The used PVA/PAA hydrogel swells with increasing pH value (Arndt et al., 1999). Immersed in a buffer solution of pH 7, the sensor measured the value of the V_{out} of ca. 54 mV. After changing from pH 7 to pH 11, the hydrogel started to swell and V_{out} rapidly increased and reached after ca. 2 h a constant value of 67 mV.

4.4 Biocompatibility of Kapton®HN foil

The biocompatibility test for Kapton used in flexible, implantable sensors showed that Kapton had only a slight influence on the cell viability regarding the control (Fig. 9). However, the decrease of 4 % in cell viability indicates still a biological compatibility of the material, whereas PET caused a decrease of 22.4 %. The latter result may have been caused by a damage of the cell culture. Nevertheless, the cell viability of more than 60 % is not classified as cytotoxic according to the norm DIN EN ISO 10993-5.

5 Conclusions

HPMA–DMAEMA–TEGDMA–EG hydrogels with different compositions and polymerization strategies were prepared, characterized and studied for their usage in dip sensors and biocompatible sensors. By increasing the amount of DMAEMA from 30 mol % (hydrogel no. 1) to 40 mol % (hydrogel no. 2), the swelling of the hydrogel became larger. The use of UV-polymerization in conjunction with the photoinitiator 2-hydroxy-4′-(2-hydroxyethoxy)-2-methylpropiophenone instead of redox polymerization led to an increase of the swelling degree. To the best of our knowledge, it was the first time this photoinitiator was used to UV polymerize HPMA–DMAEMA–TEGDMA–EG hydrogels. Advantages of photopolymerization with respect to redox polymerization are faster polymerization times, better control of the reaction process, easier patterning and geometry conservation of polymer samples, and the possibility for an in situ polymerization within the chip cavity. Glucose-sensitive hydrogel discs were fabricated by glucose oxidase diffusion into polymerized pH-sensitive HPMA–DMAEMA–TEGDMA–EG hydrogel samples. Their sensitivity was tested in both the physiological pH and glucose range, which may be of interest for blood glucose monitoring devices.

Three different hydrogel-based piezoresistive sensor setups were proposed, where two of them were implantable. The sensor assembly pictured in Fig. 2 was based on long isolated gold bond wires, and mechanical stabilization measures with medical-grade materials were performed. The sensor was coated with parylene C in order to protect the electrical components from aqueous media and to ensure the biocompatibility of the sensor assembly. The hermetic encapsulation with parylene C is widely used in microelectronics and sensor applications (Jorsch et al., 2015). The functionality of a parylene C-coated sensor was proved even after a longer storage in deionized water (Fig. 8). The flexible and partly implantable sensor setup (Fig. 3) was based on a biocompatible Kapton foil (Fig. 9) as substrate and on the flip chip technology. Results in Fig. 7 showed the promising behavior of pH-sensitive piezoresistive dip sensors with a photopolymerized HPMA–DMAEMA–TEGDMA–EG (60/40/02/20) hydrogel disc shaped like the chip cavity. As shown and discussed in the literature, a decrease in hydrogel thickness strongly speeds up the hydrogel swelling response and decreases the sensor response time (Bates, 2013; Guenther et al., 2009). In the future the response time of the presented hydrogel-based sensor setups should be improved with thinner hydrogel discs and the usage of particle-based hydrogel samples to accelerate diffusion. Furthermore, in situ photopolymerization inside the pressure chip cavity could reduce hydrogel layer thickness and improve sensor properties.

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References

Arndt, K.-F., Richter, A., Ludwig, S., Zimmermann, J., Kressler, J., Kuckling, D., and Adler, H. J.: Poly(vinyl alcohol)/poly(acrylic acid) hydrogels: FT-IR spectroscopic characterization of crosslinking reaction and work at transition point, Acta Polym., 50, 383–390, 1999.

Avula, M., Tathireddy, P., Cho, S., Rieth, L., Magda, J., and Solzbacher, F.: Implantable Biosensor Arrays Based On Smart Hydrogels And Piezoresistive Sensors For Continuous Metabolic Monitoring Procedia Engineering, Elsevier, Proc. Eurosensors XXV, 25, 1008–1011, 2011.

Bates, J.: pH-responsive hydrogel-based chemomechanical sensors designed for disposable bioreactor applications, The University of Utah, 2013.

Gerlach, G. and Arndt, K.-F. (Eds.): Hydrogel Sensors and Actuators: Engineering and Technology, Springer-Verlag, Berlin, Heidelberg, ISBN-13: 978-3-540-75644-6, 2009.

Gerlach, G. and Dötzel, W.: Introduction to Microsystem Technology – A Guide for Students, John Wiley & Sons Ltd, The Atrium, Southern Gate, Chichester, England, ISBN-13: 978-0-470-05861-9, 2008.

Gough, D. A., Leypoldt, J. K., and Armour, J. C.: Erogress Toward a Potentially Implantable, Enzyme-Based Glucose Sensor, Diabetes Care, 5, 190–198, doi:10.2337/diacare.5.3.190, 1982.

Gough, D. A., Lucisano, J. Y., and Tse, P. H. S.: Two-dimensional enzyme electrode sensor for glucose, Anal. Chem., 57, 2351–2357, doi:10.1021/ac00289a042, 1985.

Guenther, M. and Gerlach, G.: Hydrogels for chemical sensors, Hydrogel Sensors and Actuators, Springer Series on Chemical Sensors and Biosensors, Springer-Verlag, 6, Berlin Heidelberg, 165–195, 2009.

Guenther, M., Gerlach, G., and Wallmersperger, T.: Nonlinear effects in hydrogel-based chemical sensors: Experiment and Modeling, J. Intell. Material Syst. Struct., 20, 949–961, doi:10.1177/1045389X08101562, 2009.

Guenther, M., Gerlach, G., and Wallmersperger, T.: Piezoresistive biochemical sensors based on hydrogels, Microsyst. Technol., 16, 703–715, doi:10.1007/s00542-009-0978-z, 2010.

Han, I., Magda, J., Bae, Y., and Baek, S.: Biosensor, Patent, WO1999017095A1, 1999.

Han, I. S., Han, M.-H., Kim, J., Lew, S., Lee, Y. J., Horkay, F., and Magda, J. J.: Constant-Volume Hydrogel Osmometer: A New Device Concept for Miniature Biosensors Biomacromolecules, American Chemical Society (ACS), 3, 1271–1275, 2002.

Herber, S., Eijkel, J., Olthuis, W., Bergveld, P., and van den Berg, A.: Study of chemically induced pressure generation of hydrogels under isochoric conditions using a microfabricated device, J. Chem. Phys., 121, 2746–2751, 2004.

Ishihara, K., Kobayashi, M., Ishimaru, N., and Shinohara, I.: Glucose Induced Permeation Control of Insulin through a Complex Membrane Consisting of Immobilized Glucose Oxidase and a Poly(amine) Polym J, The Society of Polymer Science, Japan, 16, 625–631, 1984.

Jorsch, C., Guenther, M., Gerlach, G., Ulkoski, D., and Scholz, C. (Eds.): Implantierbares Sensorsystem mit biokompatibler Verkapselung, Forschungsgesellschaft für Messtechnik, Sensorik und Medizintechnik e.V. Dresden, AMA Verband für Sensorik und Messtechnik e.V., Berlin, Germany, doi:10.5162/12dss2015/P9.4, 2015.

Jung, D.-Y., Magda, J. J., and Han, I. S.: Catalase effects on glucose-sensitive hydrogels, Macromolecules, 33, 3332–3336, doi:10.1021/ma992098b, 2000.

Lin, G., Chang, S., Kuo, C.-H., Magda, J., and Solzbacher, F.: Free swelling and confined smart hydrogels for applications in chemomechanical sensors for physiological monitoring, Sensor. Actuator. B-Chem., 136, 186–195, 2009.

Lin, G., Chang, S., Hao, H., Tathireddy, P., Orthner, M., Magda, J., and Solzbacher, F.: Osmotic swelling pressure response of smart hydrogels suitable for chronically implantable glucose sensors, Sensor. Actuator. B-Chem., 144, 332–336, 2010.

Lucisano, J. Y. and Gough, D. A.: Transient response of the two-dimensional glucose sensor, Anal. Chem., 60, 1272–1281, doi:10.1021/ac00164a007, 1988.

Orthner, M. P., Lin, G., Avula, M., Buetefisch, S., Magda, J., Rieth, L. W., and Solzbacher, F.: Hydrogel based sensor arrays (2x2) with perforated piezoresistive diaphragms for metabolic moni-

toring (in vitro), Sensor. Actuator. B-Chem., Elsevier BV, 145, 807–816, 2010a.

Orthner, M., Buetefisch, S., Magda, J., Rieth, L., and Solzbacher, F.: Development, fabrication, and characterization of hydrogel based piezoresistive pressure sensors with perforated diaphragms, Sensor. Actuator. A-Phys., 161, 29–38, 2010b.

Schmidt, U., Guenther, M., Simon, D., and Gerlach, G.: Biochemische piezoresistive Sensoren für die Immunodiagnostik, Tagungsband des 5. Dresdner Medizintechnik Symposiums, Dresden, Germany, 2014.

Schulz, V., Gerlach, G., Günther, M., Magda, J. J., and Solzbacher, F.: Piezoresistive pH Microsensors Based on Stimuli-Sensitive Polyelectrolyte tm – Technisches Messen, Walter de Gruyter GmbH, 77, 179–186, 2010.

Wilkins, E. S.: Towards implantable glucose sensors: a review, J. Biomed. Eng., 11, 354–361, doi:10.1016/0141-5425(89)90096-4, 1989.