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A novel enzyme entrapment in SU-8 microfabricated films for glucose micro-biosensors

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Abstract
The present work investigates the utilisation of the widely used SU-8 photoresist as an immobilisation matrix for glucose oxidase (GOx) for the development of glucose micro-biosensors. The strong advantage of the proposed approach is the simultaneous enzyme entrapment during the microfabrication process within a single step, which is of high importance for the simplification of the BioMEMS procedures. Successful encapsulation of the enzyme GOx in “customised” SU-8 microfabricated structures was achieved through optimisation of the one-step microfabrication process. Although the process involved contact with organic solvents, UV-light exposure, heating for pre- and post-bake and enzyme entrapment in a hard and rigid epoxy resin matrix, the enzyme retained its activity after encapsulation in SU-8. Measurements of the immobilised enzyme’s activity inside the SU-8 matrix, were carried out using amperometric detection of hydrogen peroxide in a 3-electrode setup. Films without enzyme showed negligible variation in current upon the addition of glucose, as opposed to films with encapsulated enzyme which showed a very clear increase in current. Experiments using films of increased thickness or enzyme concentration, showed a higher response, thus proving that the enzyme remained active not only on the film’s surface, but inside the matrix as well. The proposed enzyme immobilisation in SU-8 films opens up new possibilities for combining BioMEMS with biosensors and organic electronics.

Keywords: SU-8 films; Biosensor; Microfabrication; Immobilisation; Glucose oxidase entrapment; BioMEMS.
1. Introduction
In the field of BioMEMS there is an urgent need for new materials with customised topographic and chemical properties. The negative photoresist SU-8 has found a large number of attractive applications as a fundamental building material in the construction of various analytical tools (Shaw et al., 1997; Lorenz et al., 1998). SU-8 is extensively utilised in the design and manufacturing of micro- and nano- mechanical structures for MEMS and BioMEMS applications such as actuators and electrostatic sensors, biosensors, micromoulds, microfluidic devices or packing applications (Bashir, 2004; Ainslie and Desai, 2008).

SU-8 which is a negative tone epoxy, based on the Epikote resin which, together with a photoinitiator, is dissolved in an organic solvent. The cured layer of SU-8 forms a highly cross-linked matrix of covalent bonds, which results in a hard glass-like coating (Dellmann et al., 1998; Lee et al., 2003). This material constitutes a cost-effective solution for the production of fine-patterned, mechanical resistant structures for a variety of MEMS applications because of its compatibility with different types of standard X-ray and UV lithography techniques and with different coating processes (Cremers et al., 2001).

It is well established that SU-8 has been primarily used as a material for the construction of structural elements and microfluidics components in MEMS (Weisenberg and Mooradian, 2002). BioMEMS applications require immobilisation of biomolecules on the MEMS structures (Pan et al., 2004; Fichera et al., 2007; Jeykumari and Narayanan, 2008).

In order to functionalise SU-8 for such purposes, its surface requires to be modified. In the paper presented by Joshi et al. (2007a), the epoxy-groups of the SU-8 surface were hydrolysed in the presence of sulphochromic solution. Subsequently, the surface was treated with [3-(2-aminoethyl) aminopropyl]-trimethoxysilane (AEAPS). The silanised SU-8 surface was then utilised to incubate human immunoglobulin (H1gG). The immobilisation of H1gG was proved by allowing FITC tagged goat anti-human IgG to react with H1gG. This antibody immobilisation process was employed to immobilise H1gG on microfabricated SU-8 cantilevers.

A dry method of surface modification of SU-8 was reported by Joshi et al. (2007b). The surface obtained by spin coating SU-8 on a silicon wafer, was modified by grafting amine groups using pyrolytic dissociation of ammonia in a hotwire CVD setup. Fourier transform
infrared spectroscopy was used to assess the presence of amine groups on the modified SU-8 surface and also the surface characteristics after modification. SU-8 microcantilevers were fabricated and subjected to the same surface modification protocol in order to use the above process for application in BioMEMS.

Fabrication, characterisation and development of novel read-out methods using SU-8 cantilevers for bio/chemical sensing have been demonstrated by Nordstrom et al. (2008). They reported that SU-8 is an interesting polymer for fabrication of cantilevers for bio/chemical sensing due to its simple processing and low Young’s modulus. They demonstrated examples of different integrated read-out methods and their characterisation.

Glucose biosensors, both in vivo and in vitro, represent the largest biosensor market with continuing research and development efforts for further improvement. Nearly 90% of the current biosensor market is accounted for by just one biosensor: the glucose biosensor (Reach and Wilson, 1992; Newman and Turner, 2005). The enzyme glucose oxidase which is chosen for the present work is used abundantly in glucose sensing applications (Bankar et al., 2009). It is relatively easy to test if it is still active e.g. by measuring oxygen consumption or the production of hydrogen peroxide. It is relatively stable and it is available at an affordable price. The stability and activity of immobilised enzymes in confined carrier environments depends on a number of factors such as whether the carrier is organic or inorganic, hydrophobic or hydrophilic, its chemical and mechanical stability, its porosity etc.

A recent review describes enzyme immobilisation and the various considerations that are crucial (Hanefeld et al., 2009). Enzymes can be immobilised in many different ways and immobilisation methods include simple adsorption to surfaces (e.g. via hydrophobic interaction or hydrogen bonding), immobilisation via ionic interaction, covalent binding to solid supports, cross linking of enzymes, and encapsulation (e.g. in sol-gels). As carriers for enzyme immobilisation, polymeric membranes have been used extensively since they can have various functional groups and be easily modified chemically (Abd El-Ghaffar and Hashem, 2010; Kyprianou et al., 2009). The stability and activity of immobilised enzymes in confined carrier environments depends on a number of factors such as whether the carrier is organic or inorganic, hydrophobic or hydrophilic, its chemical and mechanical stability, its porosity etc (Hanefeld et al., 2009).
Patel and his co-workers (2008) presented a self-aligned and hybrid polymer fabrication process for the development of an electro-enzymatic glucose sensor. The self-aligned fabrication process was performed using polydimethylsiloxane (PDMS) as a substrate material, SU-8 as a sensor structural material, and gold as an electrode material. During microfabrication, SU-8 demonstrated good adhesion to the PDMS. This process was employed to realise a glucose sensor with active and reference gold electrodes that were sandwiched between two SU-8 layers with contact pad openings and the active area opening to the top SU-8 layer. Glucose oxidase was immobilised within the confined active area opening in order to provide an active electrode sensing surface.

The literature survey reveals that SU-8 is one of the most promising materials with biomedical and technological applications because of its biocompatibility, stability under varying environmental conditions, and low cost. Although there has been a significant progress in the research and development of processes involving utilisation of SU-8 in the last three decades, there is no report of a successful attempt to investigate the performance and suitability of this highly researched polymer material to act as an immobilisation matrix for entrapping biomolecules in the field of biosensors which would be of great importance for cost reduction and process simplification. Up to date, the use of SU-8 in BioMEMS applications is limited to surface modification of the polymer for immobilisation of biomolecules and efforts of immobilisation of biomolecules entrapped inside and on the surface of SU-8 matrix have not been reported in the current literature. This task is pursued successfully in the present contribution and moreover the encapsulation of biomolecules is carried out in parallel with the microfabrication process in a single step.

In the present paper the concept of developing a novel BioMEMS micro-biosensor using a simple one-step microfabrication process of new polymer matrices with immobilised biomolecules is investigated. More specifically, the study aims to investigate the suitability of the widely used SU-8 polymer as a matrix for enzyme immobilisation carried out simultaneously with the microfabrication process. The ultimate objective could be the utilisation of the immobilised matrices for the development of an integrated low-cost disposable glucose micro-biosensor.
2. Experimental

2.1 Reagents

The chemicals used were obtained from the following providers: Epon™ resin SU-8 granules (Hexion) was purchased from Miller Stephenson, Danbury., Connecticut 06810 USA (http://www.miller-stephenson.com); the solvent gamma-ButyroLactone (GBL), the photoinitiator triarylsulfonium hexafluoroantimonate salts, mixed 50% in propylene carbonate, dextran clinical grade, average molecular weight 64,000-76,000, used as a solution 5% w/v in distilled water; glucose oxidase type X-S from Aspergillus niger, lyophilised powder, 100,000-250,000 units/g solid, D-(+)-Glucose ACS reagent, and 1-methoxy-2-propyl-acetate (PGMEA) from Fluka, were all purchased from Sigma-Aldrich, Buchs, Switzerland. (http://sigmaaldrich.com).

Water was de-ionized clean-room grade. Stock solutions of glucose were left to mutarotate at room temperature for 24 hours before use, and were stored in a refrigerator. In all experiments, the buffer solution used was phosphate buffer saline (PBS): 10mM Na$_2$HPO$_4$/NaH$_2$PO$_4$, 154 mM NaCl, pH 7.2, all ACS grade from Merck/VWR Nyon, Switzerland.

2.2 Instrumentation

For exposure of the SU-8 films, an UV maskaligner (Karl Suss MA 6) was used (www.suss.com). Amperometric measurements were performed using the Autolab PGSTAT-12 potentiostat in combination with the software GPES (General Purpose Electrochemical System) Version 4.9 (Metrohm Autolab, Switzerland) (www.metrohm-autolab.com).


2.3 Preparation of “customised” SU-8 solutions

The SU-8 resist was prepared ‘in-house’ as the straightforward utilisation of the commercially available SU-8 formulations was not a satisfactory route, the main reason being that, the composition of the commercially available SU-8 is not provided and therefore cannot be fine tuned for the demanding application under investigation.

The “customised” SU-8 resist was prepared by dissolving the Epon SU-8 granules in gamma-butyrolactone (GBL), and adding the photo-initiator triarylsulfonium hexafluoroantimonate.
salts. Preparation of the “customised” SU-8 resist has been described in detail in earlier publications (Psoma and Jenkins 2005; Psoma et al., 2006) with some adjustments to the new application. In all experiments, the composition was 40% SU-8 and 60% solvent (40/60) with 2.5% of UV photoinitiator.

The procedure for producing 100 mL of base solution (40/60) is briefly described as follows: Firstly, the SU-8 granules were smashed to a very fine powder (40 g), which was then mixed with GBL solvent (57.5 g) and sonicated for 1 h. Although optically clear it was found necessary to leave the solution on a chemical shaker with smooth movements at 37°C for 96 hours. The solution was stored at room temperature for at least two weeks to mature and the crystals of the SU-8 granules to be fully dissolved. Before use, an amount of 2.5 g of the photoinitiator was added to the solution and sonicated for one hour.

2.4 Microfabrication process of the SU-8 films

Silicon wafers were cleaned and de-hydrated according to standard clean-room protocol. The wafers were covered with a sacrificial layer of dextran by spin-coating a 5% w/v solution in distilled water on its surface. This sacrificial layer can be dissolved in water for easy release of the SU-8 structures (Linder et al, 2005).

The standard microfabrication process steps for SU-8 resist with enzyme formulations were as follows: 4 mL of the SU-8 mixture was spin-coated on a wafer with a starting spin speed of 500 rpm for 5 seconds and then the speed was increased to 1000 rpm for 30 seconds (the speed was adjusted in some cases, see discussion). The layers were allowed to relax for 15 minutes before soft-bake. The soft-bake step, was implemented using a programmable ramping hotplate, the temperature was slowly increased from room temperature to 65°C, where it was kept for 30 minutes; and then was allowed to decrease slowly back to room temperature. Subsequently, the resist film was exposed (250mJ/cm²) with a standard UV aligner (Karl Suss MA6) using a quartz mask. The design of the mask was selected to be a repeated pattern of a simple rectangular shape with dimensions of 5 mm x 4 mm. After exposure, a post-bake step was performed. This followed the same procedure as the soft-bake. Finally, the wafers were developed in PGMEA (approximately 60 sec) and rinsed with isopropanol, followed by distilled water and were dried using a flow of nitrogen.
After processing, the resulted SU-8 films were highly transparent, without any evidence of cracks with an approximate thickness of 5.4 μm. Eventually, the structures were released in distilled water, then collected and stored in 10mM phosphate buffer solution at a temperature of 5 °C.

2.5 Enzyme immobilisation in SU-8 films

For the experiments which required the enzyme glucose oxidase (GOx) immobilisation in the bulk SU-8 films, the same microfabrication process as described in the previous section was followed, with the main difference being in the composition of the SU-8 solution. More specifically, the glucose oxidase solution was added to the “customised” SU-8 solution (4mL) and was shaken gently trying to avoid generation of bubbles before the spin-coating step.

The enzyme was dissolved in a 10mM phosphate buffer solution pH 7.2. The standard enzyme added quantity was 10 mg of glucose oxidase (141,200 units/g) dissolved in PBS. Also different concentrations were prepared by adding 5mg and 20mg of glucose oxidase in 4mL SU-8 solution in order to investigate the influence it has on signal quantity. During the experimental work, all SU-8 films were stored in 10 mM PBS solution at 5 °C and before use they were left to reach room temperature.

2.6 Electrochemical measurements

In a first series of experiments, 40 mL of PBS was introduced in a thermostatted titration vessel (Metrohm 6.1418.110); a number of SU-8 platelets were added, ensuring that they remained in the proximity of the bottom of the vessel. Electrochemical measurements were performed using a 3-electrode setup with platinum working and counter electrode (Metrohm 6.0301.100, resp. 6.305.100) and an Ag/AgCl reference electrode (Metrohm 6.0733.100). The working electrode was polarised at +650 mV with respect to the reference electrode. After approximately 10 to 15 minutes the current had stabilised and increments of 1 M glucose in PBS were added to the solution.

A second series of tests were performed in the same thermostatted vessel at 37°C, in this case with only 500 μL of PBS solution and only one platelet of SU-8 (special care had to be taken in order not to float). The vessel was equipped with a small stirrer bar. A home-made 3 electrode cell was immersed in the electrolyte. The cell consisted of a double sided Kapton
substrate with 3 electrodes. The total length of the substrate is 23 mm, to the contact pads of the upper part, wires were soldered for further connecting, the electrodes are located at the tip (last mm) of the long lower part (16 mm long and 125 μm wide). The original metal lines were made of gilded copper, passivated with Kapton, platinum was electrodeposited on all 3 electrodes in a post process and the reference electrode was additionally coated with Ag/AgCl by a galvanostatic method. The dimensions of the working electrode were approximately 300 × 50 μm. The working electrode was polarised at +650 mV vs. the on-chip Ag/AgCl pseudo-reference electrode. Measurement was performed under constant stirring with intermittent stopping of the stirrer.

3. Results and discussions

Enzyme loaded SU-8 structures were prepared on wafer as described in the experimental part; rectangular platelets with dimensions of 5mm x 4mm were released by dissolving the sacrificial dextran layer and stored in PBS solutions at 4ºC. This shape was quite easy to be lifted from the wafer using a sacrificial layer of dextran solution. A quartz mask with a pattern of 84 rectangular shapes with the aforementioned dimensions was utilised. Standard conditions consisted of a loading 10 mg of GOx in a 4 mL formulation of SU-8, and a spinning speed of 1000 rpm. These standard conditions resulted in platelets with a thickness of 5±2 μm. Different spinning speeds and concentrations were investigated. When discussing the SU-8 films below, unless stated otherwise, the films resulting from the standard conditions are referred to. Different spinning speeds would result in different thicknesses of the films.

3.1 Surface characterisation of SU-8 films

A comparison of SU-8 films with and without enzyme with an optical microscope reveals that the plain SU-8 films have a smooth surface and show interference rings visible with the naked eye, while enzyme containing layers are much rougher. The clusters of enzyme can be clearly observed. Similar images were taken with an optical interferometer and they are presented in Figure 1. Figure 1(a) and (b) illustrate a plain SU-8 film showing a roughness variation in the nanometer range. Figure 1(a) is the 3-D image of a plain SU-8 film and Figure 1(b) is a planar image of the same film. In Figure 1(c) and (d), images show a cluster of enzyme; the central red-coloured region of the surface is elevated by a few micrometers. In this figure the elevation is 2.5 μm, in other pictures (not shown) this was up to 4 μm. This pattern was found to be more or less uniformly distributed on the whole scanned surface and
was not present in the plain SU-8 films, where the mean elevation varied in the range of few nanometers.

**FIGURE 1**

In Figure 2 surface images obtained with a Scanning Electron Microscope (SEM) are illustrated. Figure 2(a) shows a plain SU-8 film, Figure 2(b) a film with immobilized enzyme. The plain SU-8 film presents a very uniform, clear and smooth surface. Actually, the spot in the picture is a speck of dirt used for focusing. From a careful observation of the film with enzyme, it can be found that clusters of enzyme molecules are formed on the film’s surface whereas the remaining surface remains uninterrupted and uniform. This observation agrees with the patterns identified in the scan images from the optical interferometer. The presence of the clusters of GOx molecules modifies the surface morphology as it is apparent in Figure 1b. The reported diameter size of GOx molecules in the literature is in the region of 8 to 15nm (Lvov et al., 1996; Ram et al., 2000). It has to be mentioned that the SU-8 films, with loaded enzyme or without enzyme, were stored in a PBS buffer solution and were well-washed in double distilled water before taking the SEM images, thus removing the PBS salts from the samples. The clusters shown on the film surface of Figure 2 have rounded-like shapes and could not be from remaining salt of the PBS solution, as salt grains look like broken and/or deformed cubes. Consequently, the observed surface irregularities are clearly from clusters of enzyme molecules.

**FIGURE 2**

3.2 Amperometric measurements of SU-8 films with immobilised enzyme

In order to assess whether the immobilised enzyme remained active inside the SU-8 matrix, an experimental investigation was carried out using an electrochemical method (amperometric detection of hydrogen peroxide) to quantify the activity of the enzyme. The most widely used method for detecting and measuring glucose in biosensor applications is the
enzymatic oxidation of glucose in the presence of oxygen which produces gluconic acid and hydrogen peroxide. In order to define the glucose concentration, the used methods usually measure the oxygen consumption or the production of either hydrogen peroxide or gluconic acid which is related to pH changes. In the present study, the above reaction was utilised to detect different levels of glucose concentration in a solution through the further oxidation of the produced hydrogen peroxide and the measurement of the associated current. Consequently, the present method is an indirect method of glucose measurement. Hydrogen peroxide can be measured amperometrically (see Section 2.6 for details). Two series of experiments were carried out with different glass cells used in the experimental setup. In general, the experimental setup consisted of a measurement glass cell, the electrodes and the computerised data acquisition system. Subsequently, the viability of the enzyme was tested by measuring its ability to produce hydrogen peroxide upon exposure to glucose.

In a first series of tests, a platinum wire working electrode was brought into contact with a platelet in a volume of 40 mL of electrolyte, with a counter and reference electrode located further on in the electrolyte. SU-8 films without enzyme were used as reference at the outset and they showed negligible variation in the observed current during the addition of glucose in the measurement cell which occurred at a time of approximately 625 seconds after the initiation of the experiment (Figure 3(a)). In addition, standard SU-8 platelets with GOx immobilised were placed close at the bottom of a glass cell. Approximately 20 to 25 minutes after initiating the measurement, the current had stabilised and 40 µl of a 1 M glucose solution was added to the buffer solution. The signal was immediately increased and stabilised after a few minutes. Subsequent additions showed an increase in the signal. In Figure 3(b) an example of a raw measurement graph is presented. It has to be mentioned that the solution was not stirred during this experiment and consequently the exact concentration is not known. In a proof of principle, the electrode was retracted from the surface of the platelets, during which the signal decreased to virtually zero current at large distance and increased upon re-approaching the platelets.

FIGURE 3
A second series of experiments were carried out under more controlled conditions in a 500 μL volume of PBS in a titration vessel fitted with a small stirrer bar. One platelet was placed into the solution and measurements were taken with a custom made miniaturised 3 electrode cell: a platinum working and counter, and an Ag/AgCl pseudo-reference electrode. Measurements were performed under constant stirring with intermittent stopping of the stirrer. After 5-10 minutes a stable current was established and 5 μL of a 1 M glucose solution was added, resulting in a concentration of approximately 10 mM. In case of the enzyme containing platelets, a continuously increasing current was observed. The increase showed a constant slope, during the duration of the measurements (2 hours). Obviously the enzyme is continuously producing hydrogen peroxide that is accumulated in the solution. As measurements are dependent upon stirring speed, stirring was stopped with 6 minute intervals for 2 minutes for taking stir-independent measurements. The signal was found to decrease and stabilise during these 2 minutes intervals. Re-starting of the stirrer (same speed as before) resulted in a signal that could be extrapolated without the turning off of the stirrer.

**FIGURE 4**

When films with encapsulated enzyme were utilised, a very clear increase in current was observed when glucose was introduced in the solution. The response was very fast (a few seconds) and also the signal was found to be proportional to the added glucose quantity. In Figure 4, the variation of current with time is presented. The step rise in current is apparent when glucose is added to the solution at certain times. The same film was tested again one week later and showed similar behaviour proving that the enzyme remains active inside the SU-8 matrix. Similar results were acquired two months later. This long term stability indicates that the entrapment of the enzyme inside the SU-8 matrix does not deactivate or destroy the enzyme, and this can be attributed to the crosslink bonding of the amino groups in SU-8 and in glucose oxidase. In addition, the capability of glucose molecules to reach the enzyme indicates porosity in the matrix, but not signs of leakage for the enzyme. Up to date there are not similar data in the literature for the SU-8 matrix to further support the above finding.
A different set of experiments were also carried out, using films with immobilised enzyme, of the same standard dimensions but of two different average thicknesses equal to approximately 6 μm and 9 μm. All other remaining parameters of the microfabrication process remained unchanged. Figure 5 presents the current variation with time where it is obvious that there is an increase in the current in response to one addition of the same glucose quantity (10 mM) in the solution.

**FIGURE 5**

In addition, it was observed that the process becomes slower as the film thickness increases, which indicates that the encapsulated enzyme in the interior matrix of the film also plays a significant role in the process as the reaction products (hydrogen peroxide) inside the SU-8 matrix move more slowly towards the surface and in the outer region. However, additional experiments need to be carried out in order to define the exact correlation between film thickness and response time as the current experimental setup is not appropriate to perform such an investigation.

This experimental analysis step proved that the enzyme remains active not only on the surface but also inside the matrix of the SU-8 microfabricated films and the process steps that were followed during the manufacturing process did not deactivate it. Nevertheless, a limited influence on its activity is expected, but it is extremely difficult to be quantified.

Additional experiments need to be carried out to address the effect of the manufacturing process on enzyme activity, in case of the described application needs to move beyond the proof-of-principal stage. Such experiments can investigate the effect of different organic solvents, pre-bake and post-bake temperatures, exposure time, porosity of SU-8 matrix etc on enzyme activity and stability.

### 4. Conclusions

The suitability of the SU-8 polymer as a biocompatible matrix capable of encapsulating biomolecules such as enzymes was proven through the encapsulation of the enzyme GOx in the SU-8 solution. The electrochemical measurements showed that the entrapped enzyme
remained active after the micro-fabrication process. A novel aspect of the present work is that the immobilisation is carried out in parallel with the microfabrication process and not afterwards as a surface modification process.

A detailed investigation was carried out using an electrochemical method in order to check the activity of the enzyme. Testing of immobilised enzyme activity inside the SU-8 matrix was carried out using amperometric detection of hydrogen peroxide in a 3-electrode setup. SU-8 films were immersed in buffer solutions and the platinum working electrode was brought in close contact with the film. Films without enzyme showed negligible variation in current during the addition of glucose, whereas when films with encapsulated enzyme were utilised, a very clear increase in current was consistently observed when glucose was introduced. A significant conclusion from this experiment is that the enzyme remains active not only on the film’s surface but inside the matrix as well. A possible explanation could be that the SU-8 film is likely to be porous allowing glucose to diffuse to the interior and approach the immobilised enzyme where oxidation occurs. In addition, hydrogen peroxide is released and diffuses to the surrounding aqueous environment where they are detected with the platinum electrode which is in close proximity to the film surface. A test which was carried out two months after production of the SU-8 films showed that the enzyme maintained similar levels of activity with the first test which took place the first day.

The proposed sensing method can be used in combination with an electrochemical measurement technique and can be combined with the infrastructure of an already existing electrochemical sensor. The integration of the developed glucose micro-biosensor offers a promising first step for the integration with organic electronics (organic LED and photodetectors) in order to produce an integrated system which can be micromanufactured in the same polymer matrix and can form a basis for the development of the next generation BioMEMS biosensors.

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References

Reach, G., Wilson, G.S., 1992. Anal. Chem. 64(6), 381A-386A.
Figure 1

(a) 

(b) 

(c) 

(d)
Figure 3

(a) Current (A) over Time (s) for SU-8 films without immobilisation of GOx.

(b) Current (A) over Time (s) for SU-8 films with immobilised GOx (after 1 week).
Figure 4
Figure 5

- 9 micrometers (600rpm)
- 6 micrometers (1200rpm)
- without GOx

Current (nA) vs. Time (min)

0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0

0 10 20 30 40 50 60 70