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Sensors and Actuators B 81 (2002) 359–368

SENSORS AND ACTUATORS B CHEMICAL

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Glucose, lactate, and pyruvate biosensor arrays based on redox polymer/oxidoreductase nanocomposite thin-films deposited on photolithographically patterned gold microelectrodes

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Accepted 11 October 2001

Abstract

Glucose, lactate, and pyruvate sensor arrays were fabricated by depositing electrostatically complexed monolayers on lithographically patterned, individually addressable, gold microelectrodes. Standard photolithographic techniques combined with metal deposition were used to fabricate gold arrays on both SiO_2/Si and flexible Mylar substrates. These gold arrays were then functionalized with a negative surface charge through chemisorption of 11-mercaptoundecanoic acid (MUA) followed by the electrostatic assembly of a nanocomposite thin-film of a cationic osmium redox polymer and anionic oxidoreductases, either glucose oxidase, lactate oxidase, or pyruvate oxidase. When tested electrochemically, glucose, lactate and pyruvate sensors exhibited analyte sensitivities of 0.26, 0.24 and 0.133 μA/(cm^2 mM) respectively. Responses to analytes proved to be linear in the physiologically relevant concentration ranges for glucose (0–20 mM), lactate (0–10 mM), and pyruvate (0–2 mM). Standard deviations between individual electrodes of ∼18% (glucose) and 20% (lactate) were determined for the enzyme electrode arrays with five array members. Furthermore, the potential problem of sensor cross-talk was investigated by subsequently testing one array member and then array members adjacent to that sensor. The response from a pair of electrodes was approximately twice than that of a single electrode, demonstrating that the individual sensors are free of cross-talk.

Keywords: Electrode arrays; Enzyme; Electrostatic assembly; Redox polymer

1. Introduction

Numerous research efforts are devoted to the development of effective biosensors for clinical applications, most often for monitoring glucose levels in fluid samples (blood or interstitial fluid) collected from the body, or for direct in vivo monitoring, by implanting in tissue or a blood vessel [1]. Most of these efforts to develop glucose sensors have focused on the entrapment of glucose oxidase (GOX) in hydrogels [2–6] behind membranes [7–9] and immobilized through an electrostatic complexation [10–12]. While less attention has been directed to the detection of lactate and pyruvate, several groups are working on developing of amperometric sensors for these analytes [13–19]. Despite an abundance of research in the area of glucose and lactate detection, numerous issues related to sensor development remain, such as enzyme deactivation, degradation of sensor materials, fouling, or an adverse tissue response in the host. Because of these issues and others, the reliability of these devices for applications such as diabetes management is questionable.

Obviously, one can improve the reliability of these devices by improving sensor materials and design to minimize the problems described above. However, another potential method of improving reliability is through the use of redundancy [20,21] as is common in applications where component reliability is paramount for safety. An array of sensors, capable of providing redundant information about the concentration of analyte, can be used rather than a single sensor device, thereby minimizing the negative effects resulting from the failure of an individual electrode. Redundancy of the sensors is important to improving reliability of the overall measurement where reliability is define as the probability of a sensor surviving for some period of time t. This point can be demonstrated by a simple calculation. For example, if $R_m(t)$ is the average sensor reliability among a group of N sensors (reliability is defined as the number of sensors functioning correctly at time t divide by the total number of sensors), then the reliability of an array of these sensors operating in parallel is $R_s(t) = 1 - [1 - R_m(t)]^N$ [22]. Thus, for an array of five

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0925-4005/02/$ – see front matter
PII: S0925-4005(01)00982-0

sensors each with a reliability of 0.75, the reliability of the entire array is $1-(1-0.75)^5$ or 0.999, which is preferable to a reliability of a single sensor. For most systems, lifetimes are distributed exponentially, i.e. $R(t) = e^{-t/\lambda}$, where λ is the sensor's mean lifetime. The most common metric for reliability is the mean-time-to-failure or MTTF, where MTTF is equal to λ. If $MTTF_s$ is defined as the mean-time-to-failure for an array of identical sensors then $MTTF_s = MTTF(1+2^{-1}+\cdots+N^{-1})$. What becomes apparent from this simple calculation is that even though each electrode in the array may be subject to the same mode of failure (e.g. fouling, enzyme deactivation), the introduction of redundancy increases the reliability of the overall measurement as compared to a single electrode measurement because each electrode is acting independently and will not fail at the same time.

Electrode arrays can be fabricated using various methods including conventional thick film technology used to create many commercially available biosensors. However, thin-film technology adopted from the microelectronics industry is another attractive approach due to ease, quality, reproducibility and low cost of manufacturing. Electrode arrays have been photolithographically microfabricated on thermally oxidized silicon [23–26], polyimide [27,28] and other insulating substrates [29,30]. Methods of immobilizing sensing components for glucose and lactate detection onto electrode arrays included electrodeposition of GOX [25,27] and the solution casting of lactate oxidase (LAX) [17]. One approach, which affords good reproducibility and control over the architecture of the deposited species, is based upon multilayer electrostatic assembly [31–35]. This scheme has been used to deposit oppositely charged redox polymers and enzymes on electrode surfaces. Specifically, ferrocene [36,37] and osmium [10,12,38–40] based cationic redox polymers stacked alternately with GOX, LAX or other enzymes resulted in the formation of working sensors.

Here, we describe the generation of multilayer nanocomposite biosensor arrays for glucose, lactate, and pyruvate on photolithographically fabricated gold microelectrode arrays. These biosensors are composed of a self-assembled, negatively charged monolayer of 11-mercaptoundecanoic acid on gold, followed by an electrostatically complexed structure of alternately stacked cationic redox polymers and anionic enzymes. The reproducibility of individual sensor response to changes in analyte concentration is measured for redundant glucose, lactate, and pyruvate sensor arrays. Finally, we demonstrated the reproducible fabrication of electrode arrays that are free of sensor cross-talk problems.

2. Experimental

2.1. Reagents

Glucose oxidase (GOX, EC 1.1.3.4, Type X-S, 128 units/mg solid from *Aspergillus niger*), lactate oxidase (LAX, 35 units/mg solid, from *Pediococcus* species), lactic acid (60% w/w in H_2O), thiamine pyrophosphate chloride (alternatively called thiamin diphosphate) 97%, and flavin adenine dinucleotide (FAD) 94% were obtained from the Sigma Chemical Co. (St. Louis, MO). Pyruvate oxidase (PYX, EC 1.2.3.3, MW 260000) was purchased from Toyobo America, Inc. (New York, NY). Ammonium hexachloroosmate(IV), 1-hexadecanethiol (C16-SH), 11-mercaptoundecanoic acid (MUA), 2,2′-azobisisobutyronitrile (AIBN), poly(4-vinylpyridine), and 2,2′-dipyridyl (bpy) were obtained from the Aldrich Chemical Co. (Milwaukee, WI). Dextrose, methanol, ethyl alcohol, ethylene glycol and acetonitrile were obtained from Fisher Scientific Co. (Pittsburgh, PA). All reagents, unless otherwise stated, were used as received. Mylar sheets (250 μm thick) were purchased from DuPont (Wilmington, DE). Phosphate buffered saline (PBS) consisted of 1.1 mM potassium phosphate monobasic, 3 mM sodium phosphate dibasic heptahydrate and 0.15 M NaCl in 18 MΩ cm deionized water (E-pure, Barnstead). Silver epoxy was obtained from Epoxy Technologies (Billerica, MA). Shipley Microposit STR1045 positive photoresist (G line), Microposit 452 Developer and Microposit Remover were purchased from MicroChem Corp. (Newton, MA). The polycationic redox polymer, poly[vinylpyridine Os(bis-bipyridine)$_2$Cl]-co-allylamine (PVP-Os-AA) was synthesized according to a procedure described previously [11].

2.2. Equipment

The equipment for electrochemical analysis included a CV-50W Voltammetric Analyzer (Bioanalytical Systems, West Lafayette, IN), a C2 Cell Stand, a Ag/AgCl reference electrode and a platinum counter electrode. The electrochemical apparatus was controlled and data acquired using a Toshiba Pentium PC. Scanning electron microscopy (SEM) was performed with a JEOL T330A electron microscope with a magnification range of 15-20,000× and a resolution of 4.5 nm. Spin coating was performed using a P-6000 spin coater (Integrated Technologies, Inc., Acushnet, MA). Soft-bake of the photoresist was performed on a Thermolyne 1900 hot plate (Barnstead/Thermolyne, Dubuque, IA). Photomasks were obtained from Advance Reproductions (North Andover, MA). Substrate metallization was performed by Lance Goddard & Associates (Foster City, CA).

2.3. Electrode array fabrication

Si wafers (two in diameter, (1 1 1), *n*-doped, 500 μm thick) were purchased from Wafer World, Inc. (St. Petersburg, Fl). The wafers were treated with a buffered oxide etch to remove any residual native oxides or organics from the surface. Subsequently, wafers were placed in a quartz boat and put in a N_2 purged oxidation furnace. Silicon dioxide was then grown under dry/wet/dry conditions at 1100 °C to the thickness of ∼5000 Å which resulted in the creation of a high-quality insulating layer on the silicon surface.

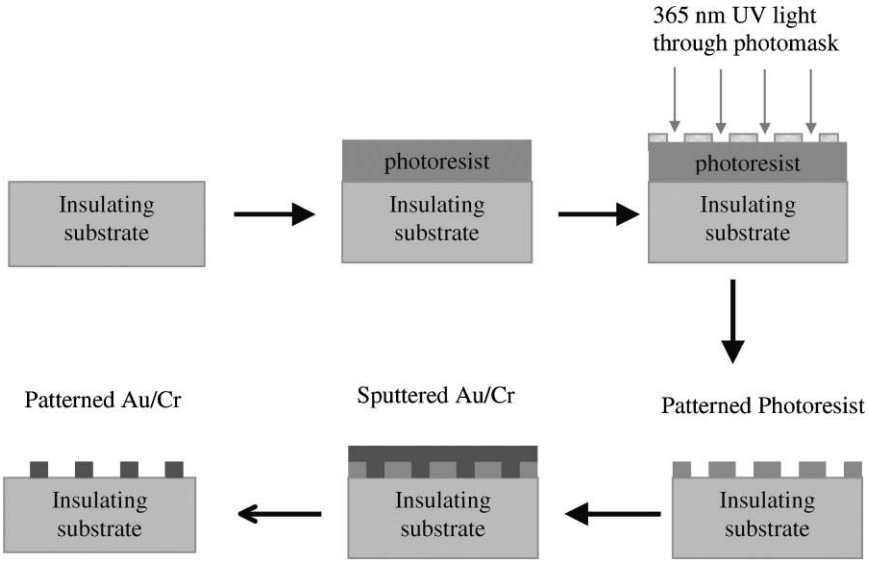

Fig. 1. Schematic of the electrode array fabrication process. A clean insulating substrate was coated with photoresist, then patterned photolithographically and developed. The patterned substrate was then metallized and lift-off used to create the electrode array.

Alternatively, Mylar was used as a flexible insulating platform for electrode manufacturing. Mylar was cut into square pieces and cleaned with ethanol prior to coating with photoresist.

The major steps for manufacturing gold electrodes on both thermal oxide and Mylar are presented in Fig. 1 and are briefly outlined below. First, a positive photoresist (Shipley Microposit STR1045) was deposited onto the substrate

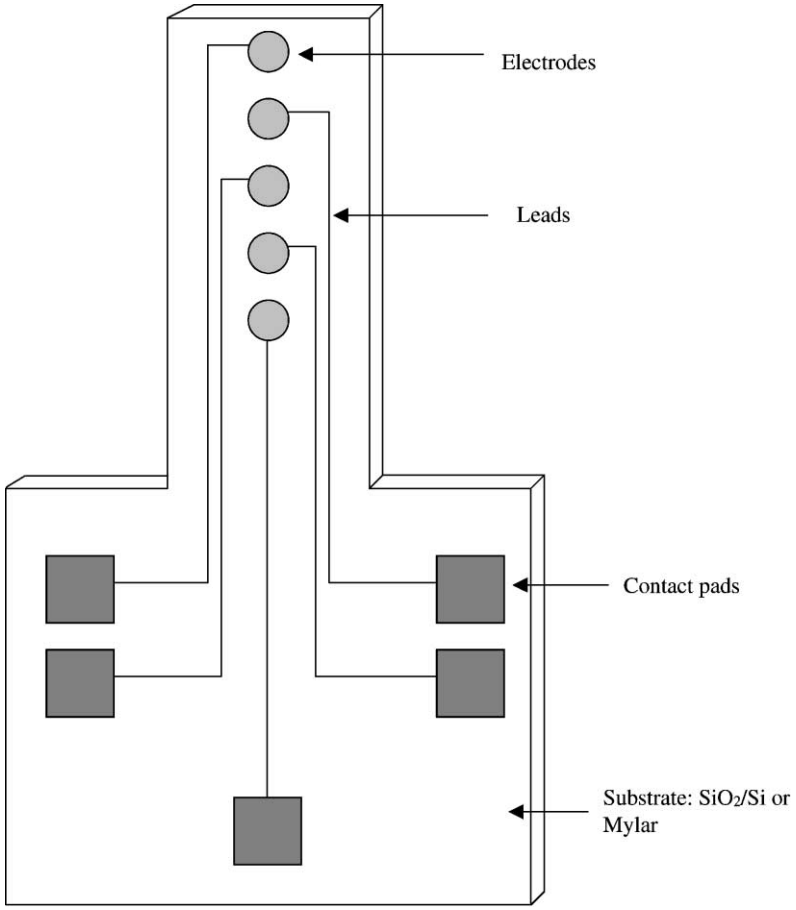

Fig. 2. Schematic of the electrode array design. Electrodes were either 50 or 500 μm in diameter with 10 μm wide leads and 6.25 mm^2 contact pads.

surface, spin-coated at 4000 rpm and soft-baked at 100 °C. The substrate was then brought into contact with a mask, exposed to 365 nm, 300 mW/cm^2 UV light for ∼0.5 s and subsequently placed in a developer solution (Microposit 452) for 3–4 min to remove portions of the photoresist that were exposed to UV light. Photoresist patterns on SiO_2 or Mylar surfaces were then sputter coated with a 200 Å adhesion layer of chrome, followed by deposition of 1000 Å layer of gold (Lance Goddard Associates, Foster City, CA). The photoresist was subsequently stripped (Shipley Microposit Remover), lifting-off chrome and gold from all non-patterned areas. This resulted in a spatially distinct pattern of gold, consisting of an array of five electrodes and thin gold lines leading to contact pads. Disk shaped gold electrodes were either 50 or 500 μm in diameter with leads of 10 μm and 2.5 mm × 2.5 mm square contact pads. A schematic of an electrode array is shown in Fig. 2. Finally, wires were attached to contact pads by soldering or use of a silver epoxy.

2.4. Nanocomposite thin-film deposition

A detailed description of the redox polymer and enzyme deposition process is presented elsewhere [11]. In brief, electrode arrays were functionalized with a negative surface charge by immersing in 1 mM MUA (ethanol) for 20 min. The substrates were washed with ethanol and dried under N_2. The arrays were then alternately exposed to a polycationic 10 mg/ml solution of PVP-Os-AA (shown in Fig. 3) for ∼20 min and a polyanionic solution of GOX (10 mg/ml in PBS), LAX (0.7 mg/ml in PBS), or PYX (25 mg/ml in PBS) for ∼40 min. In between immersions, the substrates were rinsed with 0.1 M PBS and dried under flowing N_2. In a typical deposition procedure, five layers were assembled, these layers included MUA and two layers each of redox polymer and an enzyme. To prevent redox polymer and enzyme deposition on the electrode leads, photoresist or another polymeric material may be used to mask off the leads, and thus, prevent modification to these surfaces.

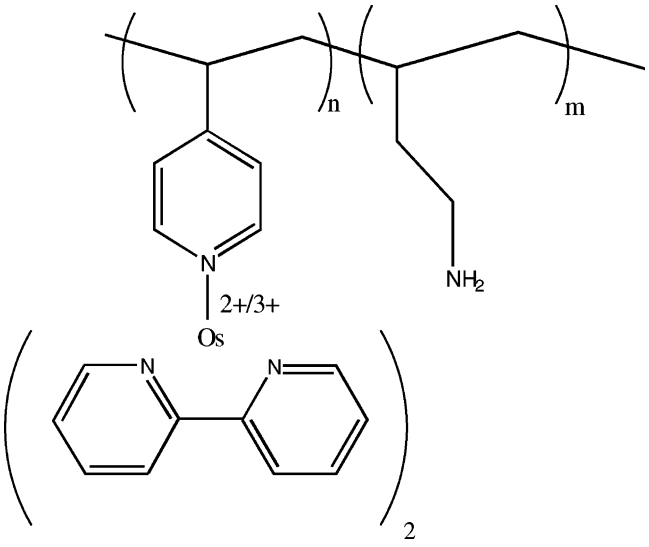

Fig. 3. Structure of the osmium redox polymer used in the development of electrostatic nanocomposites: poly[4-vinyl pyridine Os(bis-pyridine)$_2$Cl]-co-allylamine.

2.5. Electrochemical characterization

Array members were tested in a cell of volume approximately 1 ml containing 0.1 M PBS degassed with N_2 (15 min prior to start of experiment, for duration of experiment) and also containing Pt counter and Ag/AgCl reference electrodes. Degassing with N_2 agitated the solution, provided convection and created oxygen-free environment. Cyclic voltammetry, from 0 to 500 mV (vs. Ag/AgCl) at a scan rate of 20 mV/s was performed to characterize sensor response to increases in glucose, lactate, or pyruvate concentration. In the case of pyruvate, 0.2 mM of thiamine pyrophosphate and 10 μM of FAD was added to the PBS solution as these factors are necessary for PYX activity.

3. Results and discussion

Here we describe the use of microfabricated redundant gold electrode arrays with electrostatically assembled sensing components for the measurement of glucose, lactate, and pyruvate. After the fabrication of a microelectrode array using thin-film technology, the electrodes were functionalized, first with a negative surface charge, and subsequently with a cationic redox polymer and an anionic enzyme. The responses and variability of individual array electrodes to changes in analyte concentration were established. In addition, responses of multiple array members linked together are compared to that of a single array member to demonstrate that the array members do not suffer from signal crosstalk.

3.1. Electrode array fabrication

An SEM of a representative microelectrode array is shown in Fig. 4. Each of the five individually addressable array members of the electrode arrays was 50 μm in diameter while each lead was 10 μm wide. The array members appeared to be smooth with well-defined edges. No discontinuities were visible in the leads or array members. As expected the microfabrication process described earlier proved to be very reproducible as numerous defect-free electrode arrays of the same geometry were fabricated on SiO_2 and Mylar. The adhesion of deposited gold electrodes onto the substrates was strong. Mylar substrates could be flexed or sonicated without any delamination or damage to the electrode arrays. Such flexibility is useful in an implanted device. If the device were rigid, the movement of tissue near the device could result in increased tissue damage and a prolonged wound healing response.

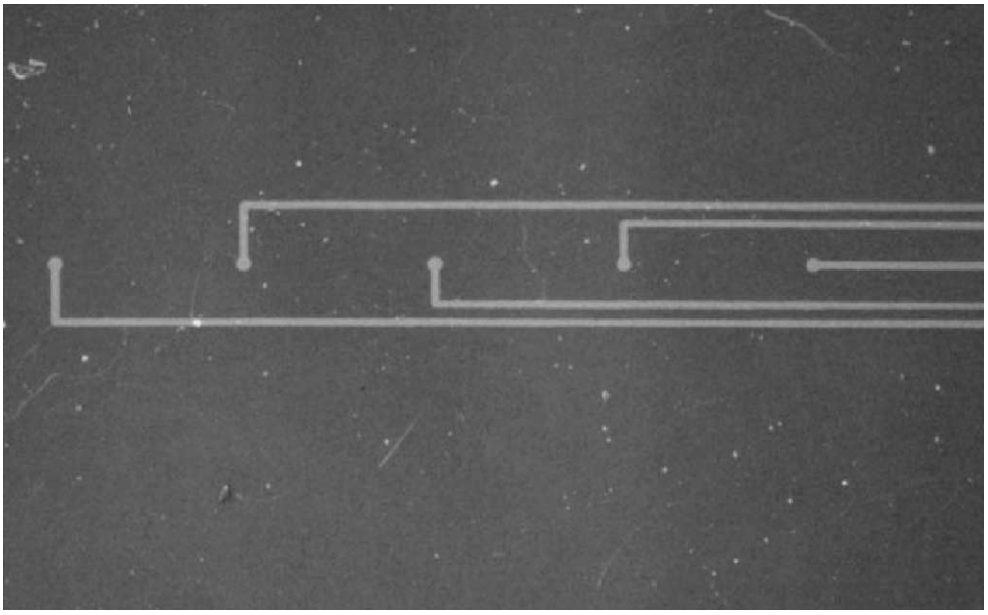

Fig. 4. Scanning electron micrograph of a gold microelectrode array on SiO_2/Si substrate.

The placement of the array members on the substrate is another important issue. If electrodes are densely packed they may mimic behavior of one large electrode of an area equal to the sum total of the areas of the individual array members [41]. Such behavior may occur when $d < 2r_0$ where d refers to the distance between array members and r_0 corresponds to the radius of the electrode. On the other hand, electrodes in loosely packed arrays with $d \gg 2r_0$ exhibit independent responses. The largest and most spatially compact electrode arrays generated in this work have electrodes with a r_0 of 250 μm and a d of 1000 μm. The inter-electrode distance, is therefore, sufficient to prevent overlay of diffusion layers, as it is two times greater than $2r_0$. Hence, it is likely that the electrodes will function independently with no cross-talk present.

3.2. Nanocomposite thin-film deposition

The scheme used for the fabrication of these sensors is based upon electrostatic attraction between a polycationic osmium redox polymer and a polyanionic enzyme, GOX, LAX, or PYX. First, the electrode surface was functionalized with MUA where the thiol end group was chemisorbed to gold. The carboxylic acid end group of MUA presented the solution–electrode interface with a net negative charge, since with a pK_a of 6.5 the acid group is at least partially deprotonated at a pH of 7.4 [42]. Polymers modified with organometallic osmium complexes are known to be polycationic [43] at neutral pH and can, thus, be electrostatically bound to the negatively functionalized surface. Subsequent electrostatic binding of anionic GOX with an isoelectric point (IEP) of 3.0, LAX with an IEP of 4.6 [44] or PYX with an IEP of 4.3 was then possible to the positively charged redox polymer layer. This process was then repeated as desired to deposit multiple layers.Electrochemical activity of the sensors

The electrochemical activity of nanocomposite thin-film sensors was verified by cyclic voltammetry. A set of representative voltammograms shown in Fig. 5(a) was obtained in the absence of substrate for a two complementary layer system (polymer/GOX/polymer/GOX). Anodic and cathodic peaks were observed at potentials of ∼250 and ∼210 mV (scan rate of 20 mV/s) respectively, resulting in a peak separation of ∼40 mV. This demonstrated successful deposition of the redox polymer and subsequent electron transfer in the polycationic redox polymer and between multilayers in the structure. Electron transfer is very likely facilitated by the high level of intercalation hypothesized to exist between the layers [42,45]. However, the relatively large peak splitting for these thin-films was very likely the result of the presence of a dielectric (i.e. the enzymes) between the layers of electroactive polymer. Other researchers working with electrostatically assembled redox polymer-based biosensors have reported similar results [10,46]. Indeed, cyclic voltammetry of a thin-film containing similar number of sensing layers (4), but incorporating PYX instead of GOX has a peak splitting of 50 mV as one can see from Fig. 5(b). The difference between peak splitting for glucose and pyruvate sensing films may be attributed to a less stable secondary structure of a PYX tetramer [19,47] which may have lead to aggregation and increased insulation between the redox polymer layers. In addition, the peak splitting increased from 50 to 70 mV when the number of complementary layers was increased from 2 to 5 (see Fig. 5(b)). This observation, corroborated by other reports describing similar modes of assembly of sensing films [40] further confirming that the enzyme acts as a dielectric between the electroactive polymer layers.

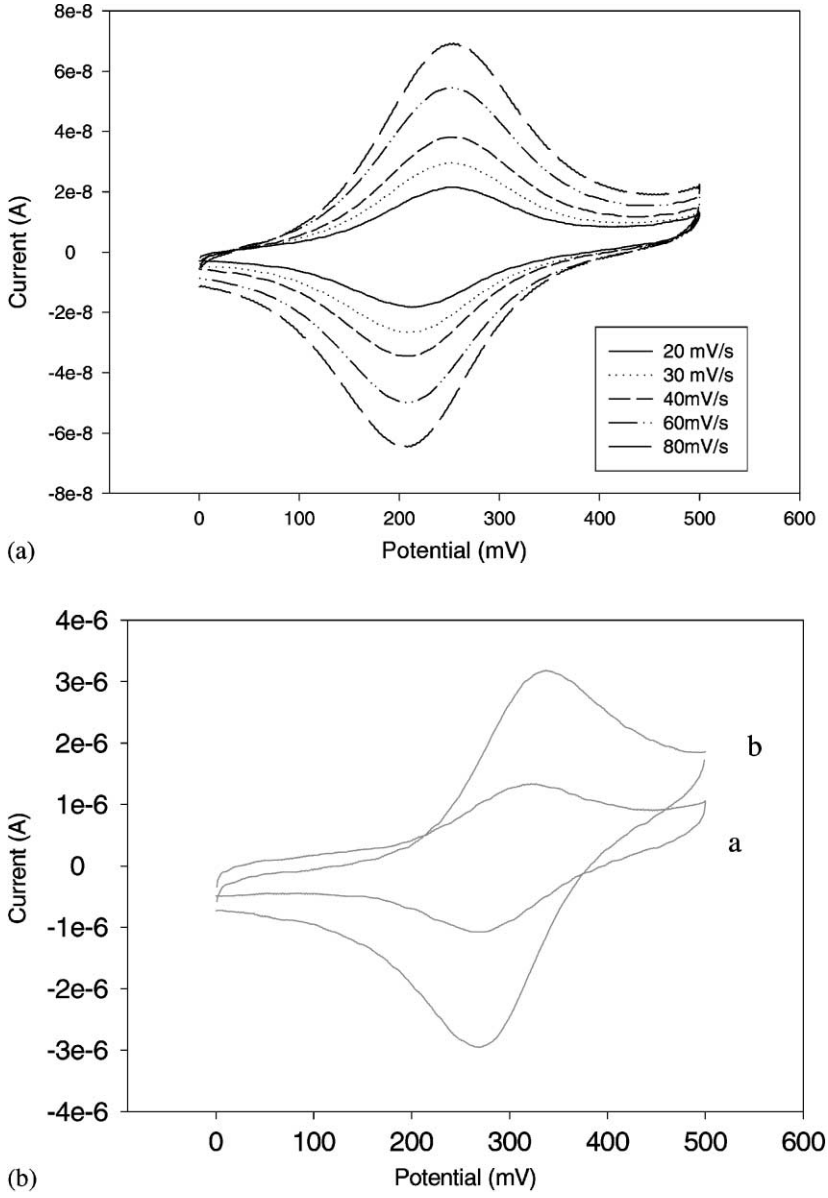

Fig. 5. (a) Cyclic voltammograms from 20 to 80 mV/s from one array member functionalized with five layers total (MUA/RP/GOX/RP/GOX), where RP is the redox polymer. (b) Cyclic voltammograms of a redox polymer/pyruvate oxidase nanocomposite thin-film electrode consisting of two complementary layers a and five complementary layers b at a scan rate of 20 mV/s.

As scan rates were varied from 20 to 80 mV/s, the peak current was found to be roughly proportional to the scan rate, as one would expect for such a thin surface confined film. These two factors point toward reversibility and stability of electron transfer within the multilayer thin-film. In addition, peak height and splitting did not change appreciably when tested repeatedly for up to three weeks (stored dry at 4 °C) indicating that the electrostatically attached redox polymer was not free to diffuse.

3.3. Sensor response and reproducibility

The GOX catalyzed reaction scheme with Os sites on the redox polymer serving as electron relays between the enzyme and electrode surface may be expressed as follows:

$$\beta\text{-D-glucose} + \mathrm{GOX(FAD)} \rightarrow \text{gluconolactone} + \mathrm{GOX(FADH_2)GOX(FADH_2)} + 2\mathrm{M}^{2+} \rightarrow \mathrm{GOX(FAD)} + 2\mathrm{M}^{+}$$

where GOX(FAD) and GOX($FADH_2$) represent the oxidized and reduced forms, respectively of the enzyme's cofactor—flavin adenine dinucleotide (FAD) and M represents an Os redox site of the polymer in its oxidized (2+) and reduced (1+) forms. Electrons are thus transferred from the enzyme to the redox polymer, shuttled between the redox sites in a self-exchange reaction until being transferred to

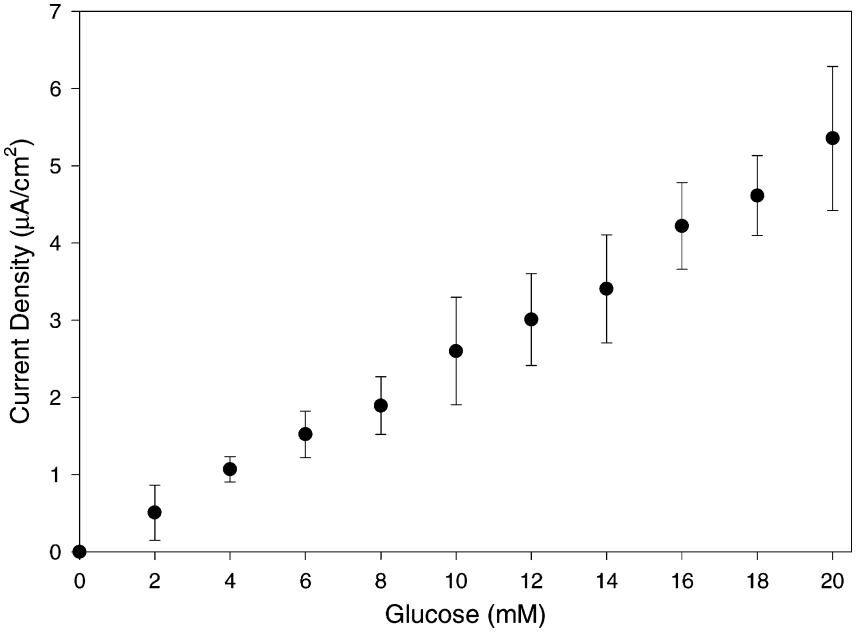

Fig. 6. Response of the current to changes in glucose concentration for a five electrode array. The percent standard deviation between electrodes was approximately 18%.

an electrode surface that is poised positive of the formal potential of the polymer. As a result, a current that is proportional to substrate concentration is created. As seen from the reaction mechanism presented above, sensors incorporating redox polymers such as those used here should be less dependent on oxygen than those based on hydrogen peroxide oxidation [48]. This fact is important for in vivo applications of a sensor, since oxygen concentration in tissue can vary.

Cyclic voltammetry was used to quantify the response of the sensors in an array and to determine the reproducibility between array members. Fig. 6 shows the average response from a five-electrode glucose sensor array. The response is linear with an R^2 value of ∼0.98 and a sensitivity of 0.26 μA/(cm^2 mM). The standard deviation between the current response of array members was approximately 18%. In addition, these sensors operated effectively when degassed with N_2 suggesting mediated electron transfer from the enzyme, through the redox polymer, to the electrode surface. Lactate sensor arrays, fabricated in the same manner as the glucose arrays, were also characterized by cyclic voltammetry. The response was linear with an R^2 of ∼0.96 and a sensitivity of ∼0.24 μA/(cm^2 mM) ($n = 5$). The standard deviation between sensors was slightly higher at approximately 20%. For both types of electrodes, the linearity of the response over the physiological range may be due to mass transfer limitations imposed by the electrostatic interactions in the redox polymer and enzyme layers similar to that seen in other systems [49]. Certainly, in the absence of these mass transfer limitations, one would expect the linear range for each type of sensor to be much lower. In the case of pyruvate electrodes, the sensitivity was 0.133 μA/(cm^2 mM) in the range of 0 to 2 mM. Beyond 2 mM, the current response to pyruvate saturated, a result of the relatively low K_m of this enzyme (0.34 mM) [47].

While strong electrostatic complexation in the films creates a mass transfer barrier to analyte sufficient to extend the linear range of each sensor, the response time of each device is still rapid. For a step change in concentration from 0 to 2 mM, the response time was less than 20 s. This relatively rapid response time is a direct result of the thinness of the multilayer films. Even though analyte diffusivity through the films may be low, the films are less than 10 nm thick and thus a steady state flux of analyte into the film can be rapidly established. Such response times were similar in magnitude to those seen in previous studies [43].

As described earlier, the placement of the array members has been addressed to minimize potential cross-talk problems between array members. Cyclic voltammetry was once again used to verify whether these array members were actually capable of functioning independently. Substrate response experiments, under identical conditions, were performed with first only one array member operational and repeated with two neighboring array elements simultaneously connected to the potentiostat. Representative calibration curves for LAX electrodes resulting from these experiments are shown in Fig. 7. Note that the area used in the calculation of current density was that for a single electrode. The result was a near two-fold increase (1.96×) in response when two array members placed next to each other were connected simultaneously as opposed to either array member individually (Fig. 8). This indicates that the array members are capable of functioning independently and at a relatively consistent response between electrodes. If one wishes to take advantage of sensor redundancy, such independence of operation is required. As was discussed earlier, the $MTTF_s$ for an array

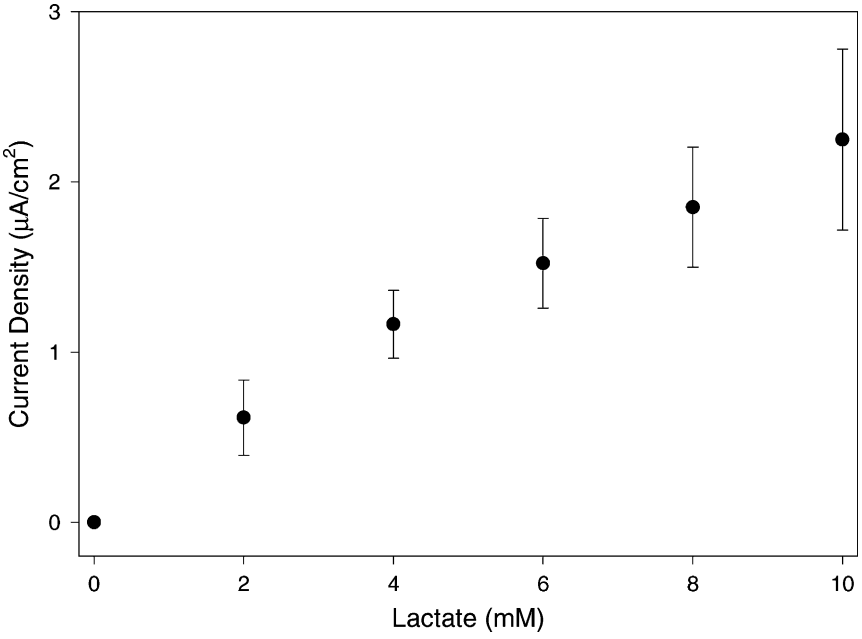

Fig. 7. Response of the current to changes in lactate concentration for a five electrode array. The percent standard deviation between electrodes was approximately 20%.

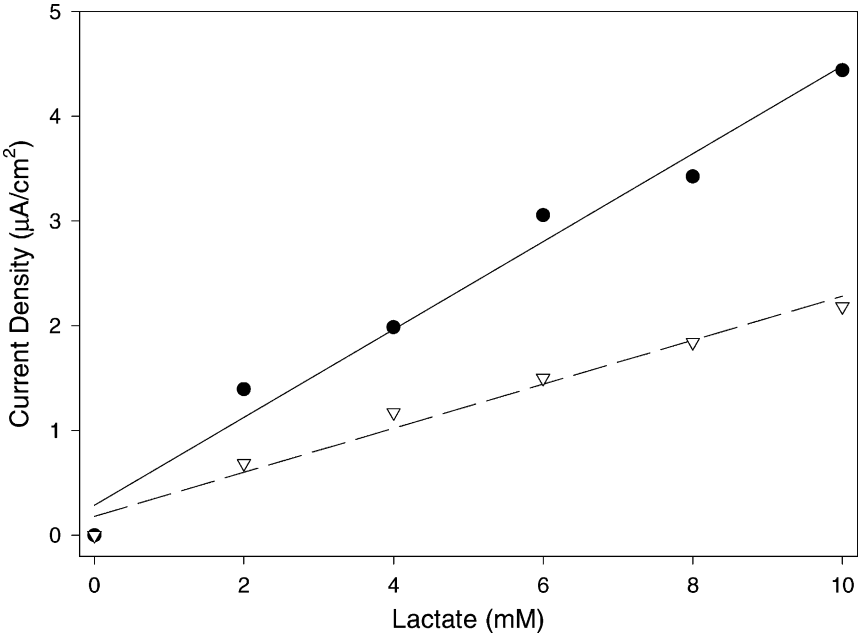

Fig. 8. Current response to changes in concentration for a lactate enzyme electrodes. One electrode (▽) was tested independently followed by the simultaneous testing of two electrodes adjacent to each other (●). The near 100% increase response when two electrodes were connected suggests that there is no electrode cross-talk present. Note that the area used in the calculation of current density was that for a single electrode.

of independently operating sensors is $MTTF_s = MTTF(1+2^{-1}+\cdots+N^{-1})$, where MTTF is the average of an individual sensor and N is the number of redundant sensors in the array. Thus, by increasing the number of enzyme electrodes from 1 to 5, the $MTTF_s$ for the system is 2.28 times greater than that of a single electrode in the array. Ultimately, the successful use of redundancy is dependent on the ability to correctly identify individual sensors with the array that have failed, perhaps using fault detection algorithms that have found use in other processes [50–52]. Such fault detection will likely be particularly challenging in vivo.

4. Conclusions

We have fabricated, on rigid and flexible substrates, gold microelectrode arrays and subsequently functionalized the array members with electrostatically complexed sensing

materials, including a cationic osmium redox polymer and an anionic enzyme (GOX, LAX or PYX). The resulting redundant sensor arrays were reproducible, with a typical standard deviation of response between array members of 18% (GOX) to 20% (LAX). Furthermore, tests were conducted to determine if cross-talk occurred between adjacent array members. Cyclic voltammetry experiments indicated no cross-talk existed between electrodes. The relative precision between multiple sensing elements suggests that rapid and cost-effective mass production may be feasible for the generation of biosensor arrays by taking advantage of silicon microfabrication schemes when coupled with simple, reproducible enzyme and redox polymer deposition schemes as described in this work. In addition to improving the physical and chemical design of electrodes, redundancy of electrodes in sensor arrays, when combined with appropriate fault detection algorithms, may improve measurement reliability in clinical applications such as diabetes management. In the future, microfabrication and sensor assembly techniques presented in this work may also be employed to manufacture multianalyte sensors on the same platform.

Acknowledgements

We would like to thank the Whitaker Foundation, Juvenile Diabetes Foundation International (1-1998-298), United States Department of the Army and the National Medical Technology Testbed, Inc. (DAMD17-97-2-7016) for funding. MVP acknowledges the support of the Alfred P. Sloan Foundation through a research fellowship. The view, opinions and/or findings contained in this report are those of the author(s) and should not be construed as a position, policy, decision or endorsement of the Federal Government or the National Medical Technology Testbed, Inc.

References

[1] E. Wilkins, P. Atanasov, Glucose monitoring: state of the art and future possibilities, Med. Eng. Phys. 18 (1996) 273–288.
[2] M.V. Pishko, A.C. Michael, A. Heller, Amperometric glucose microelectrodes prepared through immobilization of glucose oxidase in redox hydrogels, Anal. Chem. 63 (1991) 2268–2272.
[3] C.P. Quinn, M.V. Pishko, D.W. Schmidtke, M. Ishikawa, J.G. Wagner, P. Raskin, J.A. Hubbell, A. Heller, Kinetics of glucose delivery to subcutaneous tissue in rats measured with 0.3-mm amperometric microsensors, J. Am. Physiol. Soc. 38 (17) (1995) E155.
[4] E. Csöregi, D. Schmidtke, A. Heller, Design and optimization of a selective subcutaneously implantable glucose electrode based on "wired" glucose oxidase, Anal. Chem. 67 (1995) 1240–1244.
[5] K. Sirkar, M. Pishko, Amperometric biosensors based on oxidoreductases immobilized in photopolymerized poly(ethylene glycol) redox polymer hydrogels, Anal. Chem. 70 (1998) 2888–2894.
[6] C. Taylor, G. Kenausis, I. Katakis, A. Heller, "Wiring" of glucose oxidase within a hydrogel made with polyvinyl imidazole complexed with $[(Os\text{-}4,4'\text{-dimethoxy-}2,2'\text{-bipyridine})Cl]^{+/2+}$, J. Electroanal. Chem. 395 (1995) 147–153.
[7] P.D. Hale, L.I. Boguslavsky, T. Inagaki, H.S. Lee, T.A. Skotheim, H.I. Karan, Y. Okamoto, Ferrocene-modified siloxane polymers as electron relay systems in amperometric glucose sensors, Mol. Cryst. Liq. Cryst. 190 (1990) 251–258.
[8] Q. Chen, G.L. Kenausis, A. Heller, Stability of oxidases immobilized in silica gels, J. Am. Chem. Soc. 120 (1998) 4582–4585.
[9] C. Malitesta, F. Palmisano, L. Torsi, P.G. Zambonin, Glucose fast-response amperometric sensor based on glucose oxidase immobilized in an electropolymerized poly(*o*-phenylenediamine) film, Anal. Chem. 62 (1990) 2735–2740.
[10] S. Hou, K. Yang, H. Fang, H. Chen, Amperometric glucose enzyme electrode by immobilizing glucose oxidase in multilayers on self-assembled monolayers surface, Talanta 47 (1998) 561–567.
[11] K. Sirkar, A. Revzin, M.V. Pishko, Glucose and lactate biosensors based on redox polymer/oxidoreductase nanocomposite thin-films, Anal. Chem. 72 (2000) 2930–2936.
[12] E. Calvo, F. Battaglini, C. Danilowicz, A. Wolosiuk, M. Otero, Layer-by-layer electrostatic deposition of biomolecules on surfaces for molecular recognition, redox mediation and signal generation, Faraday Discuss. 116 (2000) 47–65.
[13] Y. Hu, Y. Zhang, G.S. Wilson, A needle-type enzyme-based lactate sensor for in vivo monitoring, Anal. Chim. Acta 1993 (1993) 503–511.
[14] J. Kulys, L. Wang, A. Maksimoviene, L-Lactate oxidase electrode based on methylene green and carbon paste, Anal. Chim. Acta 274 (1993) 53–58.
[15] T. Ohara, R. Rajagopalan, A. Heller, "Wired" enzyme electrodes for amperometric determination of glucose or lactate in the presence of interfering substances, Anal. Chem. 66 (1994) 2451–2457.
[16] H. Liu, J. Kong, J. Deng, An amperometric lactate sensor using tetrathiafulvalene in polyester ionomer film as electron transfer, Anal. Lett. 28 (1995) 563–579.
[17] S. Marzouk, V.V. Cosofret, R.P. Buck, H. Yang, W.E. Cascio, S. Hassan, Amperometric monitoring of lactate accumulation in rabbit ischemic myocardium, Talanta 44 (1997) 1527–1541.
[18] S.A.M. Marzouk, V.V. Cosofret, R.P. Buck, H. Yang, W.E. Cascio, S.S.M. Hassan, A conducting salt-based amperometric biosensor for measurement of extracellular lactate accumulation in ischemic myocardium, Anal. Chem. 69 (1997) 2646–2652.
[19] N. Gajovic, G. Binyamin, A. Warsinke, F. Scheller, A. Heller, Operation of a miniature redox hydrogel-based pyruvate sensor in undiluted deoxygenated calf serum, Anal. Chem. 72 (2000) 2963–2968.
[20] R. Puers, D.D. Bruyker, A. Cozma, A novel combined redundant pressure sensor with self-test function, Sens. Actuators A 60 (1997) 68–71.
[21] C.L. Stork, B.R. Kowalski, Distinguishing between process upsets and sensor malfunctions using sensor redundancy, Chem. Int. Lab. Syst. 46 (1999) 117–131.
[22] M. Modarres, M. Kaminsky, V. Krivtsov, Reliability Engineering and Risk Analysis: A Practical Guide, Marcel Dekker, New York, 1999.
[23] M. Wittkampf, K. Cammann, M. Amrein, R. Reichelt, Characterization of microelectrode arrays by means of electrochemical and surface analysis methods, Sens. Actuators B 40 (1997) 79–84.
[24] G. Buss, M. Schöning, H. Lüth, J. Schultze, Modifications and characterization of a silicon-based microelectrode array, Electrochim. Acta 44 (1999) 3899–3910.
[25] M. Koudelka-Hep, D.J. Strike, N.F. de Rooij, Miniature electrochemical glucose biosensors, Anal. Chim. Acta 281 (1993) 461–466.
[26] A. Cohen, R. Kunz, Large-area interdigitated array microelectrodes for electrochemical sensing, Sens. Actuators B 62 (2000) 23–29.
[27] J.J. Mastrototaro, K.W. Johnson, R.J. Morff, D. Lipson, C.C. Andrew, D.J. Allen, An electroenzymatic glucose sensor fabricated on a flexible substrate, Sens. Actuators B 5 (1991) 139–144.
[28] E. Lindner, V.V. Cosofret, S. Ulfer, T.A. Johnson, R.B. Ash, H.T. Nagle, M.R. Neuman, R.P. Buck, In vivo and in vitro testing of

microelectronically fabricated planar sensors designed for applications in cardiology, Fres. J. Anal. Chem. 346 (1993) 584–588.
[29] J.J. Burmeister, K. Moxon, G.A. Gerhardt, Ceramic-based multisite microelectrodes for electrochemical recordings, Anal. Chem. 72 (2000) 187–192.
[30] M.U. Nishizawa I, Microelectrode based characterization systems for advanced materials in battery and sensor applications, Electroch. Acta 44 (1999) 3629–3637.
[31] Y. Lvov, K. Ariga, I. Ichinose, T. Kunitake, Assembly of multicomponent protein films by means of electrostatic layer-by-layer adsorption, J. Am. Chem. Soc. 117 (1995) 6117–6123.
[32] Y. Lvov, K. Ariga, I. Ichinose, T. Kunitake, Molecular film assembly via layer-by-layer adsorption of oppositely charged macromolecules (linear, protein and clay) and concanavalin A and glycogen, Thin Solid Films 284-285 (1996) 797–801.
[33] S.L. Clark, M. Montague, P.T. Hammond, Selective deposition in multilayer assembly: SAMs as molecular templates, Supramol. Sci. 4 (1997) 141–146.
[34] S.L. Clark, P.T. Hammond, Engineering the microfabrication of layer-by-layer thin-films, Adv. Mater. 10 (1998) 1515–1519.
[35] S.L. Clark, M.F. Montague, P.T. Hammond, Ionic effects of sodium chloride on the templated deposition of polyelectrolytes using layer-by-layer ionic assembly, Macromolecules 30 (1997) 7237–7244.
[36] S.-F. Hou, H.-Q. Fang, H.-Y. Chen, An amperometric enzyme electrode for glucose using immobilized glucose oxidase in a ferrocene attached poly(4-vinylpyridine) multilayer film, Anal. Lett. 30 (1997) 1631–1641.
[37] J. Hodak, R. Etchenique, E.J. Calvo, K. Singhal, P.N. Bartlett, Layer-by-layer self-assembly of glucose oxidase with a poly(allylamine)-ferrocene redox mediator, Langmuir 13 (1997) 2708–2716.
[38] J. Sun, T. Wu, Y. Sun, Z. Wang, X. Zhang, J. Shen, W. Cao, Fabrication of a covalently attached multilayer via photolysis of layer-by-layer self-assembled films containing diazo-resins. Chem. Commun. (1998) 1853–1856.
[39] Y. Sun, J. Sun, X. Zhang, C. Sun, Y. Wang, J. Shen, Chemically modified electrode via layer-by-layer deposition of glucose oxidase (GOD) and polycation-bearing Os complex, Thin Solid Films 327-329 (1998) 730–733.
[40] A. Narvaez, G. Suarez, I. Popescu, I. Katakis, E. Dominguez, Reagentless biosensors based on self-deposited redox polyelectrolyte-oxidoreductases architectures, Biosens. Bioelectron. 15 (2000) 43–52.
[41] W.E. Morf, N.F. de Rooij, Performance of amperometric sensors based on multiple microelectrode arrays, Sens. Actuators B 44 (1997) 538–541.
[42] C.E. Jordan, R.M. Corn, Surface plasmon resonance imaging measurements of electrostatic biopolymer adsorption onto chemically modified gold surfaces, Anal. Chem. 69 (1997) 1449–1456.
[43] M.V. Pishko, I. Katakis, S.-E. Lindquist, L. Ye, B.A. Gregg, A. Heller, Direct electrical communication between graphite electrodes and surface adsorbed glucose oxidase/redox polymer complexes, Angew. Chem. Int. Ed. 29 (1990) 82.
[44] W. Schuhmann, T. Ohara, A. Heller, H.-L. Schmidt, Electron transfer between glucose oxidase and electrodes via redox mediators bound with flexible chains to the enzyme surface, J. Am. Chem. Soc. 113 (1991) 1394–1397.
[45] D. Yoo, S. Shiratori, M. Rubner, Controlling bilayer composition and surface wettability of sequentially adsorbed multilayers of weak polyelectrolytes, Macromolecules 31 (1998) 4309–4318.
[46] D. Laurent, J. Schlenoff, Multilayer assemblies of redox polyelectrolytes, Langmuir 13 (1997) 1552–1557.
[47] N. Gajovic, K. Habermuller, A. Warsinke, W. Schuhmann, F. Scheller, A pyruvate oxidase electrode based on electrochemically deposited redox polymer, Electroanalysis 11 (1999) 1377–1383.
[48] T. Tatsuma, K.-I. Saito, N. Oyama, Enzyme electrodes mediated by a thermoshrinking redox polymer, Anal. Chem. 66 (1994) 1002–1006.
[49] T. Chen, K. Friedman, I. Lei, A. Heller, In situ assembled mass-transport controlling micromembranes and their application in implanted amperometric glucose sensors, Anal. Chem. 72 (2000) 3757–3763.
[50] R. Dunia, S.J. Qin, T.F. Edgar, T.J. McAvoy, Identification of faulty sensors using principal component analysis, AIChE J. 42 (1996) 2797–2812.
[51] R. Dunia, S. Qin, A unified geometric approach to process and sensor fault identification and reconstruction: the unidimensional fault case, Comput. Chem. Eng. 22 (1998) 927–943.
[52] R. Dunia, S.J. Qin, Joint diagnosis of process and sensor faults using principal component analysis, Control Eng. Pract. 6 (1998) 457–469.