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REVIEW

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Electrical biochip technology – a tool for microarrays and continuous monitoring

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Abstract Based on electrical biochips made in Si-technology cost effective portable devices have been constructed for field applications and point of care diagnosis. These miniaturized amperometric biosensor devices enable the evaluation of biomolecular interactions by measuring the redox recycling of ELISA products, as well as the electrical monitoring of metabolites. The highly sensitive redox recycling is facilitated by interdigitated ultramicroelectrodes of high spatial resolution. The application of these electrical biochips as DNA microarrays for the molecular diagnosis of viral infections demonstrates the measurement procedure. Self-assembling of capture oligonucleotides via thiol-gold coupling has been used to construct the DNA interface on-chip. Another application for this electrical detection principle is continuous measuring with bead-based biosensors. Here, paramagnetic nanoparticles are used as carriers of the bioanalytical interface in ELISA format. A Si-micromachined glucose sensor for continuous monitoring in interstitial fluid ex vivo shows the flexibility of the electrical platform. Here the novel approach is a pore membrane in micrometer-dimensions acting as a diffusion barrier. The electrochemical detection takes place in a cavity containing glucose oxidase and a Pt-electrode surface. The common hydrogen peroxide detection, together with Si technology, enable precise differential measurements using a second cavity.

Keywords Electrical biochips · Microsystems · Magnetic beads · DNA array · Glucose biosensor

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Introduction

The biochip technology in biochemistry and medical diagnosis will have to provide accurate, practical, cost-effective systems and nevertheless portable devices to be used in field applications and point of care diagnosis. The early biochips were developed to be used for the measurement of products of enzyme catalyzed reactions e.g. the metabolic species glucose or lactate [1, 2]. Now the key principle of biochips is the detection and quantification of affinity binding of molecular complexes, where the transducers mainly use the evaluation of colorimetric or fluorescence signals [3] or electrochemical methods [4, 5, 6, 7, 8, 9].

By building electrical biochips in Si-technology, the realization of a compact system with high integration levels running at acceptable costs is feasible. The advantage of fully electrical chips is the intrinsic high spatial resolution and direct signal coupling of the biosensing element and the transducer. Many efforts have been made in the construction of miniaturized biosensing devices enabling electrical monitoring of metabolites as well as the evaluation of biomolecular interactions.

We present here Si-based electrical biochip arrays using an amperometric redox-recycling reaction onto interdigitated ultramicroelectrodes [10, 11], which have been arranged in array formats. The effort of a simultaneous measuring multichannel potentiostat [12] was reduced by an optimized multiplexing 16-channel potentiostat [13]. The application of those electrical biochips as DNA biochip arrays for the molecular diagnosis of viral infections demonstrates the measurement procedure. The common method of self-assembling capture oligonucleotides via thiol-gold coupling [14, 15, 16] was used to construct the array chip DNA interface.

In another application of the electrical biochips, the continuous glucose monitoring of human body fluid is shown by using a novel type of Si-micromachined sensor.

Low-density electrical microarrays

Diagnostic systems equipped with DNA or protein biochips have become a more and more important method in medical sciences. Today, the main field of applications is focused on genomic diagnosis [17]. The detection of genetic predispositions or the evaluation of pharmaceutical targets are examples [18, 19]. One application in the future of biochip assays will be the clinical diagnosis of patients for personalized medical care.

The biochips detect and quantify the interaction of complementary DNA strands to double-stranded hybrids or protein-protein interactions. The precise detection of optical signals implies the use of highly sophisticated and therefore expensive devices. In contrast, the electrochemical detection of DNA-hybridization or protein interaction with an ELISA-like test system is an inexpensive method. Many different approaches exist for the design of such electrical genosensors [20]. Some are based on physical properties of DNA, like conductivity differences due to hybridization and base stacking or changes in sterical intercalation properties [6, 7, 8].

The main principle for building up biological interfaces onto the electrical transducer is the spontaneous self-organizing binding of thiol labeled molecules onto gold surfaces [14]. These self-assembled monolayers could be chemically activated to bind DNA [15]. Thiolated DNA capture probes or protein fragments have been linked directly onto gold successfully [16, 21]. The electrical array chip technology adapted this approach.

The construction of an ELISA on chip with electrochemical detection enabled direct biosensing in an array format. The development of such electrical multiposition chips for biological detection and highly sensitive multipotentiostats enable a parallel and simultaneous analysis of complex mixtures of affinity binding molecules. These arrays consist of interdigitated gold ultramicroelectrode-arrays [12] of 0.8 µm width and 0.8 µm gap manufactured in Si technology (Fig. 1). The detection of each array position is based on redox-recycling [11] of products, generated by enzyme labeling of affinity bound complexes. The principle of the DNA-ELISA is illustrated in Fig. 2.

As a model application the identifications of Epstein-Barr virus (EBV), cytomegalovirus (CMV) and herpes simplex virus (HSV) were demonstrated on a low-density chip. This array with eight individually addressable positions connected to a multipotentiostat was used for the sequence-specific electrical detection of virus DNA. The deposition of thiolated capture DNA onto each gold electrode of the chip was performed by the use of a nanoliter-dispensing device. The whole experimental setup consisted of an in-house-made highly sensitive 16-channel multipotentiostat, a flow cell on the chip array and a computer controlled fluidic station. The whole system is short and easy to handle, which enabled transportation and in field detection. Data were directly recorded via a laptop computer.

An interrogative sequence from genomic virus DNA out of a clinical blood sample was amplified and biotinylated by a multiplex PCR [22, 23, 24]. The amplified ds-DNA was denatured and applied to the biochip surface, where the single-stranded nucleic acid targets hybridized to corresponding array positions. Thereafter positions carrying hybridized target-DNA were labeled by an ExtrAvidin alkaline phosphatase enzyme conjugate. This enzyme converted its substrate *p*-aminophenyl phosphate to the electrochemically-active product 4-hydroxyaniline. This product was measured at –50 mV and +350 mV against an external reference electrode. The quantity of bound molecules was determined by simultaneous measuring the kinetics of 3-s long periods of local current responses in a stopped flow procedure (Fig. 3).

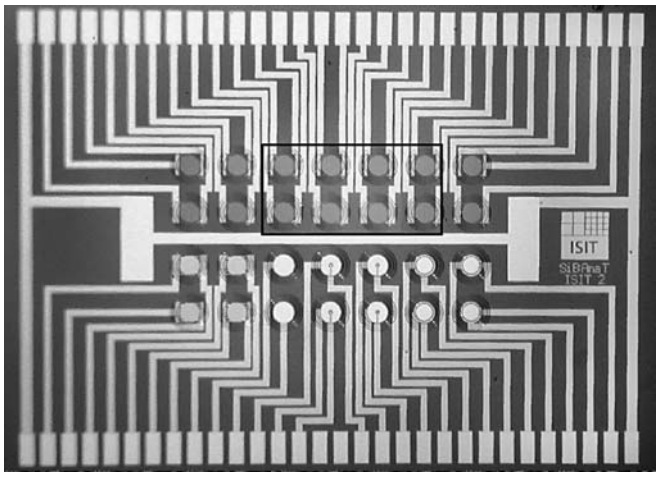

Fig. 1 Details of the electrical array-chip. The chip is equipped with 28 positions of 200 µm in diameter. Of these, 14 positions have test structures, and 14 have interdigitated gold ultramicroelectrode-arrays (IDAs)with 0.8 µm gap and 0.8 µm width. The positions used for DNA-assay are marked

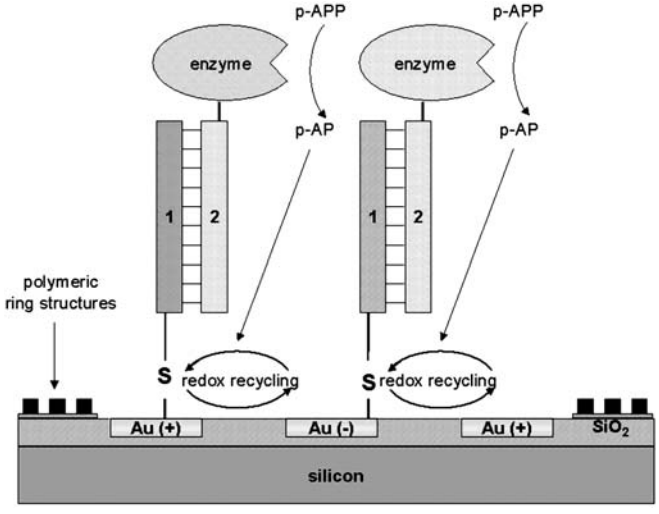

Fig. 2 Scheme of the DNA-ELISA. Target molecules bound specifically to the thiol-linked capture molecules and were than enzyme-labeled. The enzyme converted its substrate *p*-aminophenyl phosphate (*p-APP*) to the electroactive *p*-aminophenole (*p-AP*) which was measured at the electrodes

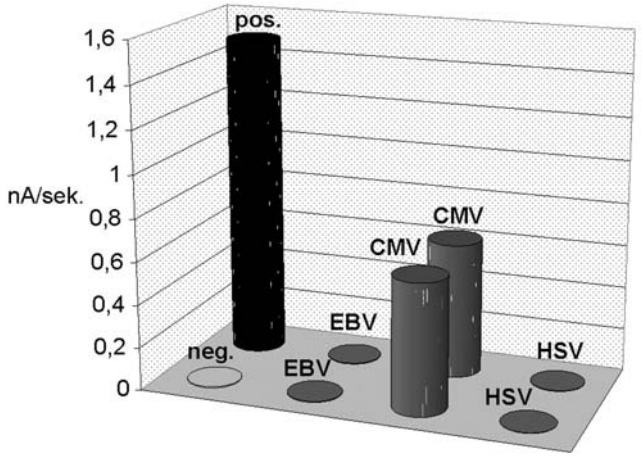

Fig. 3 Responses of the electrical virus-chip. The currents slopes were recorded simultaneously for all positions. According to the columns a cytomegalovirus (*CMV*) infection was detected. Epstein-Barr virus (*EBV*), herpes simplex virus (*HSV*)

It could be seen that the patient was CMV infected. The slope of each chip position is proportional to its target concentration which allows also the quantification of the results.

Bead-based and continuous electrochemical biosensors

Electrochemical detection principles can be used in many other formats than microarrays, and in different miniaturized devices. For several applications, microarrays may not provide the best detection platform for bioanalytics, because there might be a need for an additional separation procedure, a higher sensitivity or a continuous monitoring of analytes. Many of these alternative formats are developed to enable integrated lab-on-a-chip devices tailored to specific applications.

The use of metal nanoparticles in bioanalytical applications has recently attracted increased attention. Besides using optical properties of nanoparticles, such as changes in light scattering due to induced aggregation of particles by biomolecules' interactions [25, 26, 27] or quartz-crystal-microbalance measurements [28], electrochemical detection and quantification principles have been successfully adapted for biochip applications. Signal enhancement strategies such as silver deposition on gold particles can further increase the sensitivity and facilitate the use of nanoparticles in highly sensitive biomolecule detection [29, 30]. Wang et al. [31] used gold nanoparticles of 10 nm diameter for the direct electrochemical detection of DNA hybridization.

Magnetic beads consist of a superparamagnetic core surrounded by a polymeric outer layer suitable for the immobilization of the molecules of choice and application in electrical biochip technology.

As previously shown, not only DNA has been detected by magnetic bead-based electrochemical assays. The detection of environmental pollutants provides a wide range of targets for biosensors. Dequaire et al. [32] showed an antibody-based electrochemical sensor using a competitive immunoassay for the specific detection of herbicides. Several groups have used magnetic beads to selectively capture microorganisms from a matrix before electrochemical detection [33, 34].

The aim of many approaches in electrochemical bioanalytics is the continuous monitoring of biomolecules [35]. Here, electrical microchip technology offers the excellent sensitivity and portability of electrochemical transducers for building such biosensor systems.

Based on the described technology of Si-based electrical biochips we developed a system for semi-continuous monitoring of biomolecules in combination with magnetic beads. One application focuses on the quantification of RNA derived from microorganisms [36]. DNA capture probes on magnetic beads are used to facilitate the isolation of RNA from samples of lysed microorganisms. The capture DNA molecules are immobilized on streptavidin-coated beads by biotin modification of the probes. The capture probes and digoxigenin-labeled detection probes are simultaneously hybridized with the sample. Upon completion of the hybridization the beads are incubated with an anti-digoxigenin antibody alkaline phosphatase conjugate. Thus, captured RNA molecules on the beads are labeled with the enzyme. The beads are incubated with substrate solution containing *p*-aminophenyl phosphate. The *p*-aminophenol released by the enzyme is then detected by redox-recycling between interdigitated gold microelectrodes (Fig. 4). The highly sensitive system was able to detect of 10^{12}–10^{10} molecules of 16S rRNA within 25 min and 4 h, respectively. The most notable advantage of the system is the option of reusing the Si chip-based detection unit by separation of the biointerface onto renewable magnetic beads. The system can be further automated to provide e.g. autonomous monitoring of fermentation processes.

Glucose sensor for continuous monitoring in interstitial fluid

For many years the connection between a tight control of blood glucose level and the prevention of severe late complications due to hyperglycaemia as well as unrecognized incidents of hypoglycaemia in diabetes mellitus has been known [37, 38]. Of special focus, therefore, is the development of a continuous glucose monitoring system which is able to measure on-line glucose data as a basis for closed loop insulin application [39]. Several approaches have been followed up, of which most could not be developed as far as having a reliable system [38]. One of the main problems of continuous glucose sensors proved to be the long-term stability, which was mainly reduced by surface fouling and enzyme insensitivity by blood components and factors of the immune system [40, 41]. In particular, sensors which were implanted in the body in blood vessels or subcutaneously showed time-dependent decreasing sensitivity, resulting in incalculable calibration security [42, 43].

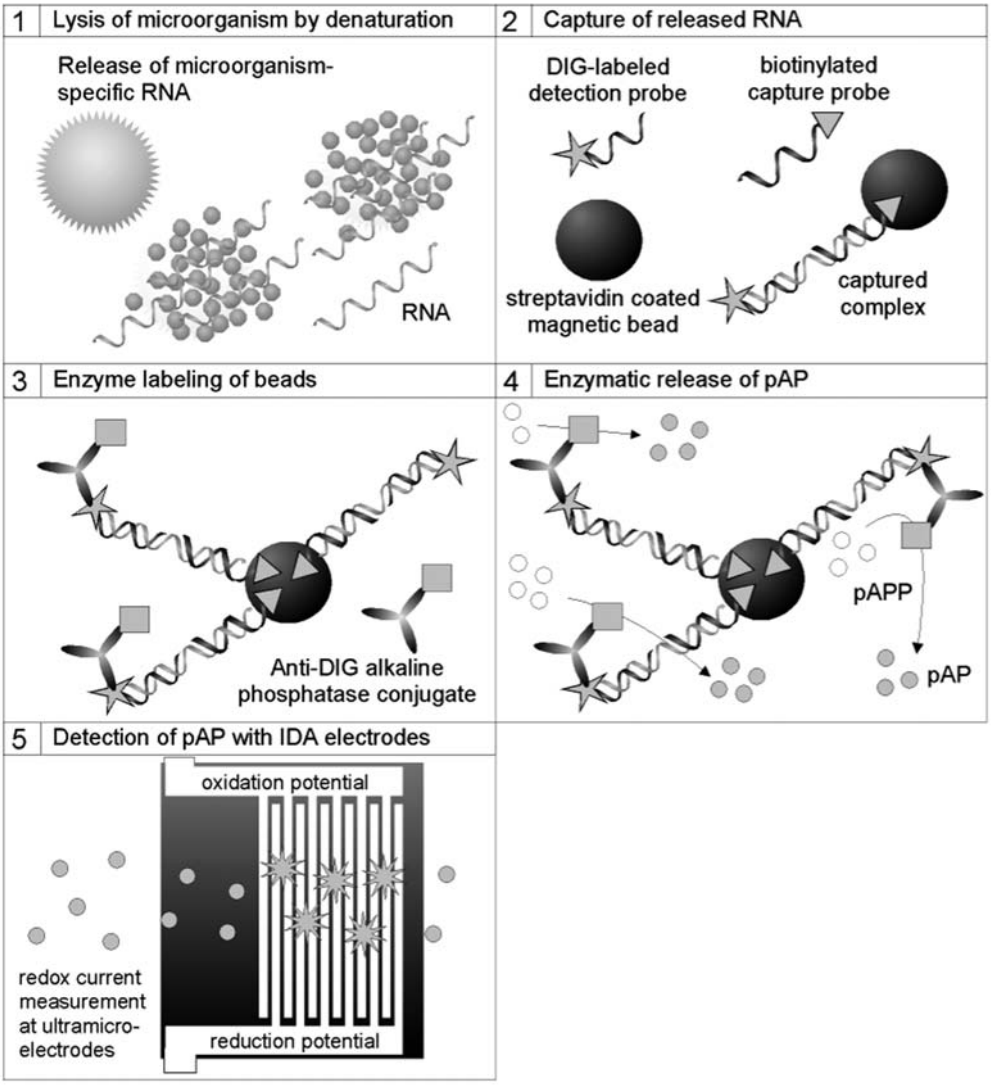

Fig. 4 Schematic depiction of the electrical detection of RNA from microorganisms by the use of magnetic beads for sample preparation and labeling. *IDA* Interdigitated gold ultramicroelectrode-arrays, p-AP *p*-aminophenole

A different approach uses glucose sensors for ex vivo application. Here, the analyte matrix is interstitial fluid, which is gained by different methods. Most common are the microdialysis [44, 45, 46], microperfusion [47, 48], and iontophoresis [49, 50] methods. The sensor systems are envisioned to be exchanged at the latest after 3 days to prevent inflammation processes, so that the demand for long-term stability is reduced to this period. In addition, the exclusion of various protein and blood components reduces in turn the danger of sensor fouling and enzyme inactivity. The disadvantage of the dislocation of the sensor outside the body is a time delay of the measurement values caused by the fluidic components for interstitial fluid sampling.

In parallel, non-invasive optical techniques have been developed, which have, due to the overlap of the glucose peak by other components, barriers to becoming a success [51]. The most developed systems, also in terms of market presence, are the glucose monitors of Minimed, Medtronic, [51] and the GlucoWatch by Cygnus [52]. Both have obtained premarket approval by the FDA.

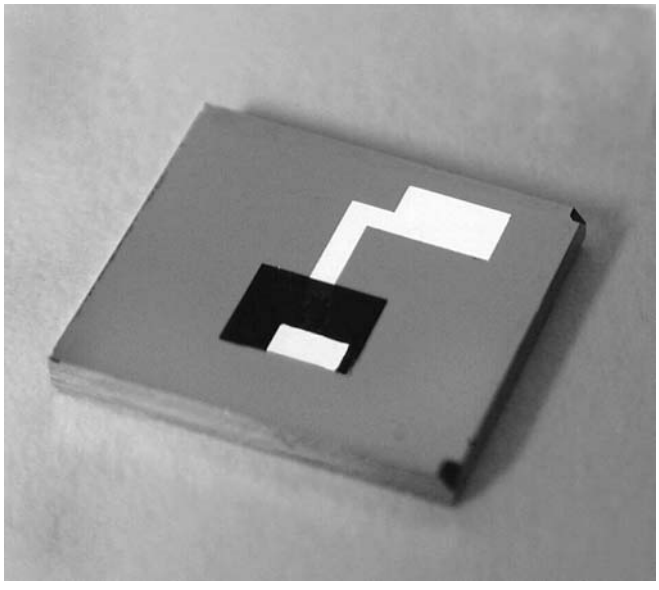

Fig. 5 Photograph of a Si cavity chip

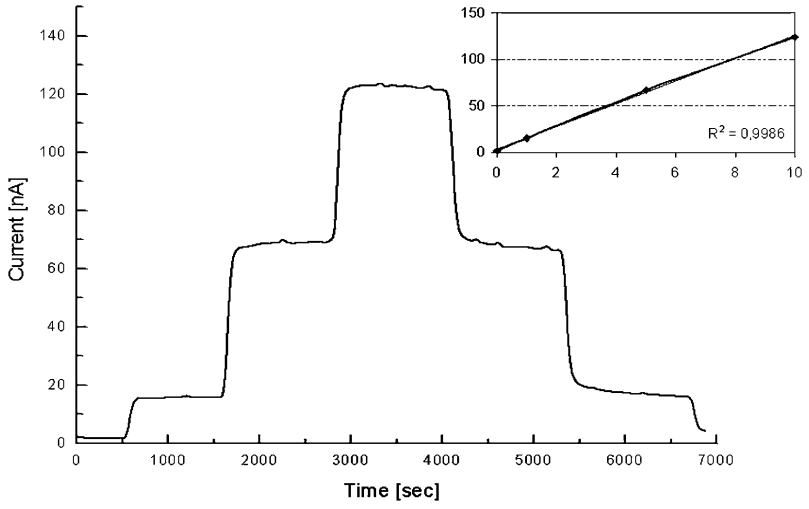

Fig. 6 Signal response at increasing and decreasing glucose concentrations (0–1–5–10–5–1–0 mM glucose in physiological buffer solution) and diagram (*upper right corner*) of the sensor current

Based on previous work [53, 54] we developed a glucose sensor for continuous monitoring in interstitial fluid (Fig. 5). The sensor consists of a micromachined Si chip, including an etched cavity and a pore membrane. Inside the cavity a platinum working electrode is situated on the cavity ground membrane, which is contacted over one edge to the chip surface. Furthermore, the enzyme glucose oxidase is immobilized inside the cavity. The sensor is integrated in a flow-through packaging embodying a sealed flow channel, the reference and counter electrodes, as well as electrical contacts. The measurement principle is the conventional enzymatic oxidation of glucose followed by electrochemical (amperometric) detection.

The advantage of this sensor configuration lies on the one hand in the pore membrane acting as a diffusion barrier, which permits control of the access of analyte to the cavity and thereby the possibility of adjusting the linear range of the sensor. On the other hand, the chip cavity functions as a semi-closed cell which means protection of the sensitive sensor components such as enzyme and electrode surface. The chip fabrication is defined by high precision Si technology and enables high calibration stability and reproducibility from chip to chip. Additionally, reference measurements with a parallel cavity layout are thereby enabled.

The Fraunhofer ISIT glucose sensor was tested in vitro as well as in vivo. In vitro, all important sensor parameters such as linear range, signal response, long-term stability, and the ability to compensate signals from interfering substances have been evaluated. The evaluation and characterization of the sensor was done under in vivo conditions in numerous clinical studies. Here, it was coupled to the microperfusion technique [47].

The sensor showed in all respects a very good performance. The linear range, which has to be between 0.5 and at least 10 mM glucose is easily reached (Fig. 6). The signal response is, at about 30 s at a pump speed of 0.5 µl/min, rather fast, and the long-term stability exceeds by at least 1 week the demand of 3 days for a replaceable extra-corporal continuous sensor. Problems by signal-interfering substances could not be assessed in interstitial fluid. If requested, a double cavity sensor can be used in which the second cavity functions as a reference sensor for effective compensation of interfering signals.

In clinical studies, the sensor has so far been tested only up to 24 h. The results of the measured interstitial glucose values correlated very well with blood glucose values, which were determined regularly as a control (Fig. 7). Due to the very rapid signal response of the sensors, the dynamics of the glucose signal and variations in the interstitial fluid recovery were followed instantly.

The cavity principle of the ISIT glucose sensor and the first performances indicate it to be a good candidate to fulfil the long-term and calibration stabilities in human body fluids.

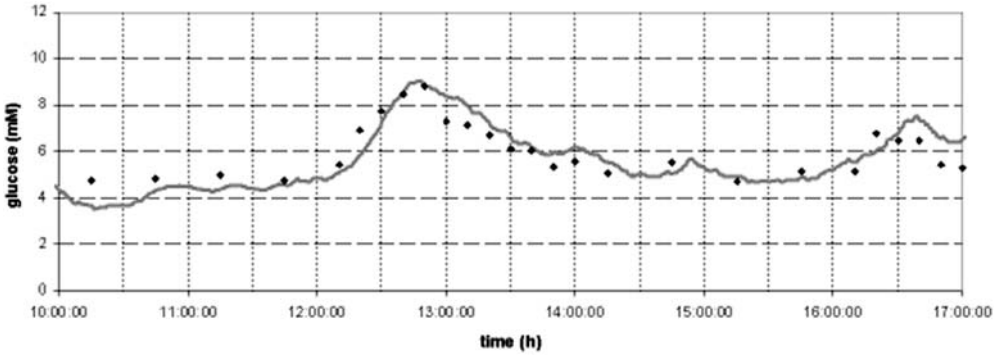

Fig. 7 Data of the ISIT continuous glucose sensor (*grey line*) measured in interstitial fluid and reference blood glucose values (*dots*)

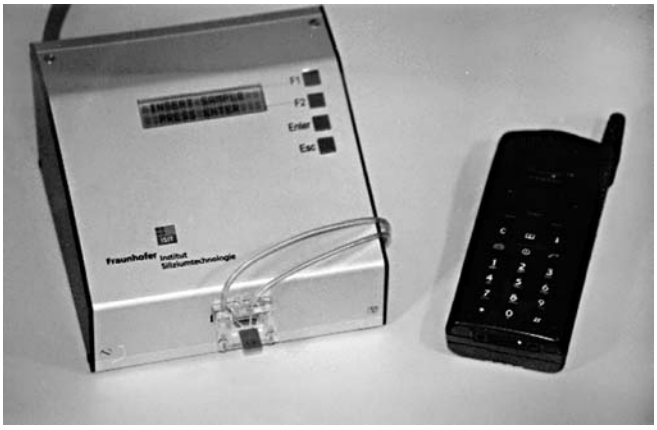

Fig. 8 Photo of an eight-channel potentiostat with flow cell and DNA-chip in front

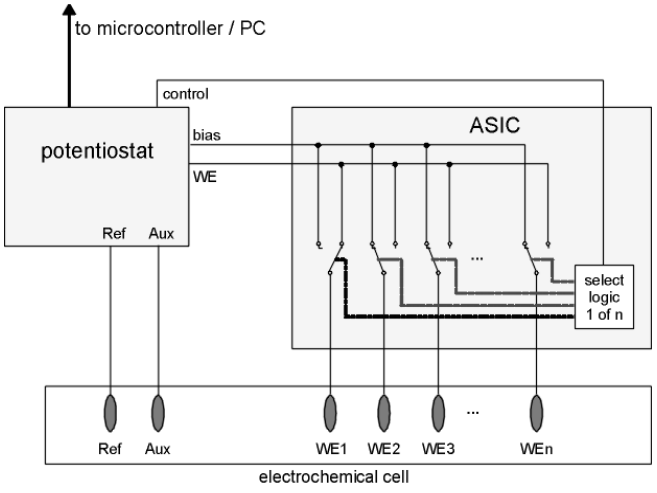

Fig. 9 Scheme of a multipotentiostat with multiplexer

Instrumentation of electrical biochips

All the applications of electrical biochips discussed here are based on electrochemical amperometric measurements, where enzyme reactions or affinity binding events cause current responses at the microelectrode constructions via production of small redox molecules as described above. Several types of instruments for the measurement of electrical biochips have been developed. These instruments, specially dedicated to the ultramicro electrodes, are microprocessor-controlled multipotentiostats with 2–32 independent measurement channels and various I/O capabilities. The telephone-sized instrument, as shown in Fig. 8, allows a direct connection with the sensor chip in the front of the device.

The most sensitive measuring procedure is the simultaneous control and measurement of all electrodes [12]. This requires individual measurement amplifier circuits for each single electrode, which have to be controlled by a powerful 16-bit microprocessor via an interface between analog and digital sections. Additionally, the processor controls the input/output of signals to actuators and to data handling (Fig. 9). Typical actuators are pumps, different kinds of valves and heating or cooling devices. A PC is connected by a serial interface (RS232). A special software on the PC, called MCDDE, allows the entry of all parameters, the starting of measurements, the collection of measurement data and the transfer of these data to a commercial data analysis software (Origin). For example, there are devices to measure 16 channels of a DNA chip with currents in the picoampere range, or other devices for two channels of glucose sensors, which typically have a two decades higher current range.

Another special instrumental development was the serial read out of multiple channels by the construction and manufacture of a high sophisticated CMOS multiplexer [13]. This was made in order to reduce the amplifier circuit boards, and thus also save power and space. The multiplexer, which was realized as an application specific integrated circuit, allows the connection of eight electrodes to one measurement amplifier. The special quality of this multiplexer is that it allows a constant potential at the electrodes at every point of the serial measurement cycle, and that the switching doesn't influence the precision of the measurement. The compromise that has to be made in this version of the potentiostat is that the measurement rate and measuring time per channel is lowered because of the serial read out.

The most recent instrumental version was realized to measure 128 or 1,024 electrodes of so-called fully electronic active biochips, which have been developed by Infineon, Munich, in collaboration with us [55]. Here a new approach in the electrical biochip technology was made. In the so-called electronic biochips, the measurement amplifiers are integrated together with a multiplexer inside the Si sensor chip in conventional CMOS-technology. The electrode configuration and the biointerface are the same as discussed above.

An advantage of the developed multipotentiostats, which were designed as a modular system of stacked electronic boards, is the flexibility that allows a free programming of all the details of biochemical assays and measurement procedures.

Conclusions

Electrical biochip technology is a suitable platform for the detection of affinity binding complexes of all biological relevant molecules and conjugates e.g. nucleic acids, proteins, and small molecules such as haptens, as well as the detection of redox active derivatives of carbohydrates as e.g. glucose or lactate.

The power of the semiconductor technology and its usefulness to extreme and precise miniaturization of the transducing elements enable the application of highly sensitive amperometric methods e.g. redox recycling with very low and competitive detection limits. The technology avoids any mechanical adjustment, as is necessary for op-

tical sensing elements, and allows the construction of robust, portable devices. Further, conventional and well-defined biochemical tools such as the ELISA technique may be adapted to multichannel sensing. The principles and general construction of the electrical biochip platform offers further integration with CMOS-circuits to fully electronic biochips and microfluidics, as well as wireless data transfer to the networks.

References

1. U.Wollenberger, R.Hintsche, F.Scheller (1995) Biosensors for analytical microsystems. In: Reichl H (ed) Microsystem technologies. Springer Verlag, Berlin Heidelberg New York pp 687–693
2. Hintsche R, Dransfeld I, Scheller F, Pham MT, Hoffmann W, Hüller J, Moritz W, (1990) Biosens Bioelectron 5:327–334
3. Vo-Dinh T, Cullum B (2000) Fresenius' J Anal Chem 366: 540–551
4. Palecek E, Fojta M, Jelen F (2002) Bioelectrochemistry 56:85–90
5. Wang J (1999) Anal Chem 71:328–332
6. Kelley SO, Boon EM, Barton JK, Jackson NM, Hill MG (1999) Nucleic Acids Res 27:4830–4837
7. Berggren C, Stalhandske P, Brundell J, Johansson G (1999) Electroanalysis 11:156–160
8. Alfonta L, Singh AK, Willner I (2001) Anal Chem 73:91–102
9. Willner I, Willner B, Katz E (2002) Rev Mol Biotechnol 82: 301–463
10. Hintsche R, Paeschke M, Wollenberger U, Schnackenberg U, Wagner B, Lisec T (1994) Biosens Bioelectron 9:697–705
11. Niwa O, Xu Y, Halsall HB, Heineman WR (1993) Anal Chem 65:1559–63
12. Paeschke M, Dietrich F, Uhlig A, Hintsche R (1996) Electroanalysis 10:891–898
13. Hintsche R, Albers J, Bernt H, Eder A (2000) Electroanalysis 12:660–665
14. Bain CD, Whitesides GM (1989) J Am Chem Soc 111:7164–7175
15. Brockman J, Frutos AG, Corn RM (1999) J Am Chem Soc 121:8044–8051
16. Kelley SO, Barton JK, Jackson NM, Hill MG (1997) Bioconjug Chem 8:31–37
17. Harris T (2000) Med Res Rev 20:203–211
18. Risch NJ (2000) Nature 405:847–856
19. Roses AD (2000) Nature 405:857–865
20. Pividri MI, Merkoci A, Alegret S (2000) Biosens Bioelectron 15:291–303
21. O´Brien JC, Jones VW, Porter MD, Mosher CL, Henderson E (2000) Anal Chem 72:703–710
22. Pozo F, Tenorio AJ (1999) Virol Methods 79:9–19
23. Yamamoto T, Nakamura Y (2000) J Neurovir 6:410–417
24. Defoort J-P, Martin M, Casano B, Prato S, Camilla C, Fert V (2000) J Clin Microbiol 38:1066–1071
25. Elghanian R, Storhoff JJ, Mucic RC, Letsinger RL, Mirkin CA (1997) Science 277:1078–81
26. Park SJ, Taton TA, Mirkin CA (2002) Science 295:1503–6
27. Weizmann Y, Patolsky F, Willner I (2001) Analyst 126: 1502–4
28. Reichert J, Csaki A, Möller R, Köhler JM, Fritzsche W (2000) Anal Chem 72:6025–6029
29. Möller R, Csaki A, Köhler JM, Fritzsche W (2001) Langmuir 17:5426–5430
30. Cai H, Xu Y, Zhu N, He P, Fang Y (2002) Analyst 127:803–8
31. Wang J, Xu D, Kawde AN, Polsky R (2001) Anal Chem 73: 5576–5581
32. Dequaire M, Degrand C, Limoges B (1999) Anal Chem 71: 2571–2577
33. Perez FG, Mascini M, Tothill IE, Turner AP (1998) Anal Chem 70:2380–2386
34. Che Y, Li Y, Slavik M (2001) Biosens Bioelectron 16:791–797
35. Wang J (2002) Acc Chem Res 35:811–816
36. Gabig-Ciminska M, Holmgren A, Andresen H, Barken KB, Wümpelmann M, Albers J, Hintsche R, Breitenstein A, Neubauer P, Los M, Czyz A, Wegrzyn G, Silfversparre G, Jürgen B, Schweder T, Enfors SO (in press) Biosens Bioelectron
37. The Diabetic Control and Complications Trial Research Group (1993) N Engl J Med 329:997–1036
38. Gerritsen M, Jansen JA, Luttermen JA (1999) Neth J Med 54: 167–179
39. Shichiri M, Sakakida M, Nishida K, Shimoda S (1998) Artif Org 22:32–42
40. Rhemrev-Boom RM, Tiessen RG, Jonker AA, Venema K, Vadgama P, Korf J (2002) Clin Chim Acta 316:1–10
41. Gerritsen M, Jansen JA, Kros A, Vriezema DM, Sommerdijk NAJM, Nolte RJM, Lutterman JA Van Hövell SWFM, Van der Gaag A (2001) J Biomed Mat Res 54:69–75
42. Kerner W, Kiwit M, Linke B, Keck FS, Zier H, Pfeiffer EF (1993) Biosens Bioelectron 8:473–482
43. Kerner W (2001) Exp Clin Endocrinol Diab 2:341–346
44. Meyerhoff C, Bischof F, Sternberg F, Zier H, Pfeiffer EF (1992) Diabetologica 35:1087–1092
45. Maran A, Crepaldi C, Tiengo A, Grassi G, Vitali E, Pagano G, Bistoni S, Calabrese G, Santeusanio F, Leonetti F, Ribaudo M, Di Mario U, Annuzzi G, Genovese S, Riccardi G, Previti M, Cucinotta D, Giorgino F, Bellomo A, Giorgino R, Poscia A, Varalli M (2002) Patients Diab Care 25:347–352
46. Jungheim K, Wientjes KJ, Heinemann l, Lodwig V, Koschinsky T, Schoonen AJ (2001) Diab Care 24:1696–1697
47. Schaupp L, Ellmerer M, Brunner GA, Wutte A, Sendlhofer G, Trajanoski Z, Skrabal F, Pieber TR, Wach P (1999) Am J Physiol 276:E401–408
48. Trajanoski Z, Brunner GA, Schaupp L, Ellmerer M, Wach P, Pieber TR, Kotanko P, Skrabal F (1997) Diab Care 20:1114–1121
49. Tamada JA, Garg S, Jovanovic L, Pitzer KR, Fermi S, Potts RO (1999) JAMA 282:1839–1844
50. Tierney MJ, Jayalakshmi Y, Prris NA, Reidy MP, Uhegbu C, Vijayakumar P (1999) Clin Chem 45:1681–1683
51. Mastrototaro J (1999) J Pediatr Endocrinol Metab 12:751–758
52. Tierney MJ, Tamada JA, Potts RO, Jovanovic L, Garg S (2001) Biosens Bioelectron 16:621–629
53. Hintsche R, Neumann G, Dransfeld I Kampfrath G, Hoffmann B, Scheller F (1989) Anal Lett 22:92175–2190
54. Scheller F, Pfeiffer D, Hintsche R, Dransfeld I, Nentwig J (1989) Biomed Biochim Acta 48:891–896
55. Thewes R, Hofmann F, Frey A, Holzapfl B, Schienle M, Paulus C, Schindler P, Eckstein G, Kassel C, Stanzel M, Hintsche R, Nebling E, Albers J, Hassmann J, Schülein J, Goemann W, Gumbrecht W (2002) ISSCC Digest of Tech. Papers 350–351 and 472–473