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Biosensors: Unique Tools in Pharmaceutical and Biomedical Sciences

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Summary : *Biosensors are, by definition, sensing devices comprising a biological component (enzyme, antibody, animal or plant cell, oligonucleotide, lipid, microorganisms, etc.) intimately connected to a physical transducer (electrode, optical fiber, vibrating quartz, etc.). This dual configuration permits the determination of a great variety of compounds of pharmaceutical interest and it allows drug interaction studies with the immobilized biocomponent. Ideally, biosensors should be readily implemented and allow low reagent and energy consumption. Enzyme-based biosensors can be applied in the pharmaceutical industry for bioprocess control (in bioreactors,etc.), by following in real time the formation or consumption of analytes of interest such as glucose, glutamate, amino acids, lactate, etc. Affinity biosensors are suitable for high throughput screening of bioprocess-produced antibodies and for highly selective and sensitive immunoassays. Enzyme-based biosensors are especially well suited as home testing devices for glucose, lactate, uric acid, and cholesterol or in hospitals for bedside testing, emergency control, in surgery (e.g. lactate monitoring), r urea monitoring during dialysis treatments, etc. In clinical laboratories miniaturized arrays of biosensors are dedicated to the control of many physiological parameters (glucose, urea, uric acid, creatinine, acetylcholine.) and for a variety of drug analysis by immunoassays with the antibody or the antigen immobilized onto the transducer. Current research efforts in the biosensor field are oriented towards the use of living cells immobilized in biochips. This configuration comprises several different microelectronic sensors and biosensors sensitive e.g. to pH, temperature, impedance, oxygen, glucose, for a multiparametric cellular monitoring for advanced stages of drug screening. Of equal new interest are the oligonucleotide-immobilized biosensors for interactions studies between the surface linked DNA and the target drug or for hybridisation studies. This short review summarizes the state of the art in biosensors dedicated to applications of pharmaceutical and biomedical interests.*

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The development of biosensors started in the early sixties with the concept of enzyme immobilized electrodes for the determination of analytes of physiological interest such as glucose and urea[1]. The bioconversion of those molecules into readily detectable species by enzymes, attached in close proxi-

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mity to a physical transducer, allowed the determination of physiological compounds otherwise difficult to analyse.

A variety of biological species and transducers may be considered for biosensor construction. Ideally a biosensor should be small sized and easy to use. It should allow reagentless assays, offer fast and reversible responses with high sample throughput and exhibit storage and operational stability.

Several varieties of configurations have been envisaged since the early description of a glucose oxidase (GOx) electrode. The biological component may be immobilized physically or chemically onto the transducer. Chemical links allow more stable devices but care must be taken during the chemical steps in order to avoid denaturation of the biocomponent. Additionally, a thin membrane may be advantageously coated onto the biosensor for improving the selectivity, extending the linear range of analysis or in order to minimize surface fouling by interfering molecules.

Interest in biosensors development went on relatively smoothly till the commercial launching, in the nineties, of two new biosensor concepts. One consisted in miniaturized electrode strips for glucose assay in a microdroplet of whole blood, this was thoroughly reviewed in[2]. The other successful innovative biosensor combined microfluidics and biosensing, allowing real time and automated affinity analysis (e.g. for antigen-antibody assays) using an original optical sensing concept based on surface plasmon resonance (SPR). The latter is a sophisticated and expensive instrumentation based on laser excitation, self assembled monolayers and microfluidics. This biosensor is preferably applied in the pharmaceutical industry for research purposes or in bioprocess monitoring e.g. in antibody screening[3]. Interestingly, however, recent works showed the usefulness of biosensors based on SPR for the decentralized testing of drug residues (hormones, antibiotics) in foods of animal origin[4].

The concept of hand-sized biosensor devices using "one shot" strips attracted immediate commercial success. In fact, it opened the way of testing at sites or in a satellite laboratory, decreasing the laboratory turnaround time (sample transport → laboratory test → transfer data → action). Many sectors of our society are highly demanding for such easy to use measuring devices namely in medical diagnostics, for fast screening of illicit drugs, in environmental pollution control, in food an beverage industries, in biological and chemical warfare, in sport doping testing, for space applications etc.

Electrochemical (EC) based biosensors are most often described and are currently facing major successful marketing[5]. Most EC biosensors operate amperometrically (monitor the current at a constant potential) and use relatively stable enzymes (oxidase) for the determination of glucose, galactose, lactate, ethanol, glutamate, sulphite, acetate, hydrogen peroxide or a combination of enzymes for sucrose (invertase + mutarotase + GOx) for acetylcholine (acetylcholine esterase + choline oxidase) for creatinine (creatinine amidohydrolase + creatine amidinohydrolase + sarcosine oxidase). These instruments are automated under microprocessor control and are supplied by e.g. Yellow Spring Inst. (www.YSI.com), Nova Biomedical (www.novabiomedical.com), Trace (www.trace-ag.de), Chemel AB (www.chemel.com), Applisens (applisens@applikon.com) etc.. The transducer generally detects liberated hydrogen peroxide or alternatively a redox mediator is inserted in the biolayer for electron transfer acceleration between the enzyme and the electrode. The fragile nature of the immobilized biocomponent imposes, however, frequent calibrations and biomembrane renewal. Depending of the enzyme, the stability of the sensing probe allows for several weeks of operation before replacement of the sensing layer.

In clinical analysis newly compact instruments with arrays of biosensors inserted on line offer automated and high throughput multisensing capabilities. Progress in microfluidic and chip technology has allowed the launching of i-STAT System (www.i-stat.com) for blood analyses. This hand-held clinical

analyser features amperometric, potentiometric and conductimetric sensors for blood electrolytes, urea, ammonium ions, glucose, pO_2 and hematocrit determinations. The i-STAT biomedical instrument is worth to mention because of its ingenious and miniaturised concept and because it offers a multianalyte sensing capability. The sensing part comprises, in a chip format (single use), miniaturised electrodes for several physiological ions such as potassium, sodium, calcium, and biosensing parts for urea (potentiometric biosensor) and glucose (amperometric biosensor). Especially attractive is the fact that the chip allows in situ calibration before the assay[6].

Amperometric biosensors based on carbon composite electrodes have been described in the early eigthies. The attractive concept is that the enzyme can be readily immobilised in the electrode matrix by simple mixing with the electrode components i.e., graphite and binding agent (paraffin, Teflon, epoxy etc.). The resulting biosensor may be shaped as a cartridge for multiple use or as a single use strip by screen printing the "biopaste". For ensuring high electron transfer efficiency between the biocomponent and the electrode, a redox mediator is advantageously comprised in the paste. Several commercial attempts in developing carbon composite cartridges have failed. Yet, one company has recently launched a biosensor showing improved robustness by using a solid binding matrix for enzyme immobilisation. Amperometric biosensors for glucose, fructose, ethanol, malate and lactate analysis in drinks using the carbon composite concept are available (Biofutura,Torino,Italy)[7,8]. Most employed though are the screen printed biosensors, based also on graphite dispersed in a suitable polymer matrix, where the miniaturized sensing part allows microvolumes of samples to be analyzed and the sensing tip is disposable. Biosensor instability was elegantly solved, as already said, by the development of such single use strips. The latter are generally prepared by screenprinting the entire three electrodes system and microdispensing the enzyme and any additional reagent/membrane onto the strip. Such technique allows also antibody or antigen immobilisation for immunobiosensors preparation[9].

EC biosensors exploiting potentiometry with an ammonium selective electrode are generally dedicated to urea determination in hospital care control[10]. Others exploit the glass pH electrode with immobilized penicillinase for bioprocess monitoring during penicillin production[11]. Trends in miniaturized biosensors consists in the development of microsensor chips comprising different microelectronic sensors namely Ion Selective Field Effect Transistor (ISFET), oxygen microelectrodes etc. and immobilized living cells[12]. Such multiparametric systems allow changes in the extra cellular acidification rate to be measured on-line and non-invasively. Variations of cell adhesion, cell morphology and intercellular junctions are detectable by impedance measurements with interdigitated microelectrodes. Such microchips may be useful for the detection of both cell metabolic and cell physiological responses to drugs. Several microbial biosensors have been described[13]. A very unique application of a potentiometric sensor exploits living cells retained on an ISFET. Here the phenomenon of extracellular acidification is monitored as a measure of the viability and growth of the cells. The biosensor is based on a combination of a light adressable potentiometric sensor (LAPS) with a micro-flow chamber containing the living cells. The environmental acidification by the cells is related to their physiological status. This biosensor allows the rapid and sensitive detection of functional responses upon receptor stimulation in real time[14].

Glucose biosensors are dominating the biosensor market and this can be explained by the commercial availability and good stability of the enzyme glucose oxidase (GOx), by the relatively high physiological concentration of glucose and of course by the huge market for glucose sensing. About 90 % of the consumer diagnostic market is blood glucose monitoring, the rest is largely home testing for pregnancy and blood coagulation. Electrochemical biosensors for personal diabetes management using test strips allowing accurate blood glucose determination are

nowadays flourishing. Even though blood samples as low as three microliters can be analysed with test strips, non-invasive biosensors for glucose have attracted substantial research efforts. The GlucoWatch (Cygnus, Inc., Redwood City, Cal. USA) painlessly measures blood glucose every 20 min. for up to 12 hrs at a time. Glucose is collected through intact skin by iontophoresis via application of a direct electric current. Once in the gel disc at the biosensor, the glucose reacts with GOx to form hydrogen peroxide, which is measured amperometrically. Currently, the device cannot be a substitute for a traditional glucose test done by pricking a finger to draw blood since it should be perfected for frequent glucose monitoring[15].

Immunobiosensors with EC, optical (SPR, fluorescence) or mass sensitive (piezoelectric microbalance) transducers are gaining extensive research interest (www.nanogen.com[16,17]. As for enzymes, antibodies or antigens may be readily immobilized onto transducers. The highly selective molecular recognition may be monitored directly due to a change in the physicochemical parameter at the sensing tip (affinity probes) or indirectly by detection of the labelled immunoagent. Alkaline phosphatase, horseradish peroxidase and glucose oxidase are the most popular enzyme labels for immunoassays. Immunosensors for protein A, digoxin, theophylline, salmonellas, IgG etc., have been described. A general problem with immunosensors is the difficulty to reversibly regenerate the sensing surface. To solve such limitation, single use strip immunosensors have also been described for field portable devices[18].

Amperometric biosensors have been also applied for in vivo sensing since they may be readily miniaturized[19,20]. The sensing probe can either be implanted or connected on-line with a microdialysis sampling unit[19] for the monitoring of localized biochemical events and for the determination of physiological parameters (glucose[21], glutamate[20] lactate, hydrogen peroxide, acetylcholine…). By the appropriate casting of thin membranes onto the biosensors tip, high selectivity and biocompatibility may be achieved. Substantial progresses in pharmacology have been observed thanks to the use of implanted biosensors, but efforts still remain to be carried out before commercial viability of such devices.

Protein, oligonucleotide or DNA chips (biochips) are at present readily available and extensively used. With a $ 500 million-per-year market, the DNA probe technology is the fastest growing in vitro diagnostic market. It is forecast to grow 25% per year during the next few years. Infectious disease detection is the largest used tests for sexually transmitted diseases, tuberculosis and pneumonia. Tests for human immunodeficiency virus and hepatitis virus are available now. In addition to the very high sensitivity, the testing times have decreased from more than a day to a few hours. Research in DNA sensors is especially attracting much interest using chips coupled to fibre optics waveguides or CCD camera detectors[22-24]. Commercial devices exploit generally a fluorescent probe for monitoring the hybridization reaction. Considerable progress is observed in portable optical devices for drug screening, sequencing by hybridization, cell screening, epitope mapping (www.Biomerieux.com , www.nanogen.com, www.affymetrix.com)

Alternative reading, in protein array chips, consists of revealing the analyte retained on the chip by the use of surface-enhanced laser desorption (MALDI)/ionization for determining its molecular weight (www.ciphergen.com). Such systems allow for comparison of protein profiles from multiple samples simultaneously to rapidly detect changes in protein expression levels. We should point out though, that following the biosensor definition[25], DNA and protein chips with optical reading such as those mentioned above are, strictly speaking, not a biosensor since the biocomponent is not directly linked to the physical transducer. Yet research underway is focused on immobilizing microarrays of oligonucleotides directly onto the optical fibre tip[26]. Much hope is also expected from DNA microarrays with electrochemical detection by the recent launching of an "electronic" DNA chip by the company Motorola

(www.motorola.com)[27]. When a single immobilized DNA strand encounters a complementary partner in a sample, it will hybridise. This event can be directly detected by electrooxidation (or reduction) of a redox label accumulated at the immobilized DNA duplex or by monitoring a change of the electrical property at the biosensor interface (capacitance, impedance)[27-29]. For example, conductivity changes can be observed during hybridisation with the nucleic acid probe immobilized within a conducting polymer[30]. Other configuration uses lipid bilayers onto gold electrodes with entrapped ion-channel protein acting as an ion-channel-switching biosensor. Such biomimetic devices may serve both for immunosensors and for DNA biochip development (www ambri.com.au). Protein and DNA microarrays are generally based on gold electrode since gold is readily shaped into different microconfigurations and functionalized with thiomolecules. The latter, chemisorbed as a monolayer on gold, serve for subsequent oligonucleotide (protein) attachment and for minimizing surface fouling phenomena by large molecules during the assay. EC DNA chips are also suited for studying DNA base damage in vitro by measuring the effect of the xenobiotic on the oxidation signal of guanine[31].

Biosensors offer real innovative concepts and bring new investigation opportunities for the pharmaceutical research and health care domains (see www.globind.com for ordering information on biosensors in medical diagnostics). Their uniqueness relies on the presence of a sensitive immobilized biological component. Yet marketing success is dominated by glucose biosensors and DNA biochips. The commercial launching of different biosensors is in fact not in quantitative relation with the exponential growth of the number of publications in the field. Many explanations can be provided for this situation and probably the main reasons are:

1- The limited market for the considerable research investment. This is related to lack of adapted legislation for specific sample testing and to reduction in reimbursement levels for in vitro diagnostic testing.

2- The reluctance of some organisations for product launching approval. This is observed in the medical sector which estimates that the risks of self testing and diagnostic by patients may be worst than good for the patient essentially because of the absence of data interpretation (risks of false positive and negative responses because of non-appropriate testing etc.) and absence of advice by the professional: such situations could be dramatic e.g. in the case of HIV, cancer, viruses or in cholesterol testing etc.

3- The lack of comparison of biosensor performances with conventional testing instruments.

4- The concern of ethical aspects, especially in relation with genotyping.

In conclusion, since the first description of a EC biosensor in 1962, tremendous progress has been observed in biosensor development both at a technological and theoretical level. This has been achieved thanks to progresses observed in other research areas such as in material sciences, microtechnology, membrane technology and molecular biology. Biosensors of the future will continuously need to benefit from progresses in other scientific disciplines. This overview summarizes some EC biosensors and their use, other applications such as in military, aerospace and food testing is currently under intensive research as well.

References

1. Kauffmann J-M , Guilbault GG. Enzyme electrode biosensors: theory and applications, in Bioanalytical Applications of Enzymes, Suelter CH, Kricka L (eds) J. *Wiley & Sons,* Inc.N.Y. vol 36 pp.63-114, 1992.
2. Magner E. Trends in electrochemical biosensors, *Analyst 123,* 1967-1970,1998.
3. Quinn JG, O'Kennedy R. Transduction platform and interfacial design of biosensors for "real time" biomolecules interaction analysis, *Anal.Letters* 32, 1475-1517, 1999.
4. Baxter AG, O'Connor MC, Haughey SA, Crooks, SRH, Elliott CT. Evaluation of an immunobiosensor for the on-site testing of veterinary drug residues at an abattoir. Screening for sulfamethazine in pigs, *Analyst*,

124, 1315-1318, 1999.

5. Zhang S, Wright G, Yang Y. Materials and techniques for electrochemical biosensor design and construction, *Biosensors & Bioelectron.* 15, 273-282, 2000.
6. Jacobs, E.Vadasdi E, Sarkozi, L. Colman N, Analytical evaluation of i-STAT portable clinical analyzer and use by nonlaboratory health-care professionals, *Clin. Chem.,* 39 1069-1074, 1993.
7. Svorc J, Miertus S, Katrlik J, Stred'ansky, M, Composite Transducers for Amperomertic biosensors. The glucose sensor, *Anal.Chem.,* 69. 2086-2090, 1997.
8. Miertu S, Katrlík M, Pizzariello A, M Stred'ansk_ M, vital J, vorc J. Amperometric biosensors based on solid binding matrices applied in food quality monitoring, *Biosensors & Bioelectron.,* 13, 911-923 ,1998.
9. Pemberton RM, Hart JP, Mottram TT. An electrochemical immunosensor for milk progesterone using a continuous flow system, *Biosensors & Bioelectron.,* 16, 715-723, 2001.
10. Jurkiewicz M, Solé S, Almirall J, Garcia M, Alegret S, Martinez-Fabregas E. Validation of an automated urea analyser used in the continuous monitoring of hemodialysis parameters *Analyst,* 121, 959-964 , 1996.
11. Höbel W, Papperger A, Polster J. Penicillinase optodes: substrate determinations using batch, continuous flow and flow injection analysis operation conditions. *Biosensors &Bioelectron.* 7, 549-557, 1992.
12. Ehret R, Baumann W, Brischwein M, et al. Multiparametric microsensor chips for screening applications, *Fres. J. Anal. Chem.* 369, 30-35, 2001.
13. D'Souza SF. Microbial biosensors, *Biosensors & Bioelectron.,* 16, 337-353, 2001.
14. Owicki JC, Parce JW. Biosensors based on the energy metabolism of living cells: the physical chemistry and cell biology of extracellular acidification. *Biosensors & Bioelectron.* 7, 255-272 , 1992.
15. Wu C, Kersten B, Chen Q, Li J, Jagasia P. QC test for non invasive glucose monitoring system, *Electroanalysis* 13, 117-122 , 2001.
16. Schult K, Katerkamp A, Trau D, Grawe, F, Camman K, Meusel M . Disposable optical sensor chip for medical diagnostics: new ways in bioanalysis, *Anal.Chem.,* 71, 5430-5435, 1999.
17. Ghindilis AL, Atanasov P, Wilkins M, Wilkins E. Immunosensors: electrochemical sensing and other engineering approaches. *Biosensors & Bioelectron.,* 13, 113 -131, 1998.
18. Lee K-S , Kim T-H, Shin M-S, Lee W-Y, Park J-K. Disposable liposome immunosensor for theophylline combining an immunochromatographic membrane and a thick-film electrode, 380 17-26 , 1999.
19. Koudelka-Hep M, van der Wal P.D. Microelectrode sensors for biomedical and environmental applications, *Electrochim.Acta* 45, 2437-2441, 2000.
20. Kulagina NV, Shankar L., Michael AC, Monitoring glutamate and ascorbate in the extracellular space of brain tissue with electrochemical microsensors, *Anal. Chem.,* 7, 5093-5100, 1999.
21. Pravda M, Kauffmann J-M, Michotte Y. Development of an on-line electrochemical biosensor for glucose determination in rat brain using microdialysis sampling, *Electroanalysis* 12, 912-916, 2000.
22. Liu X, Tan W. A fiber-optic evanescent wave DNA biosensor based on novel molecular beacons, *Anal. Chem.,* 71, 5054-5059, 1999.
23. Vo-Dinh T, Alarie JP, Isola N, Landis AL, Wintenberg AL, Ericson MN. DNA biochip using a phototransistor integrated circuit. *Anal. Chem.,* 71, 358-363, 1999.
24. Yang JM, Bell J, et al., An integrated microlaboratory for biological agent detection with DNA and immunoassays, *Biosensors & Bioelectron,* 17, 605-618, 2002.
25. Thévenot DR, Toth, K, Durst RA, Wilson GS. Electrochemical biosensors: recommended definitions and classification, 16, 121-131, 2001.
26. Zhang G, Zhou Y, Yuan J, Ren Y. A chemiluminescence fiber-optic biosensor for detection of DNA hybridization, *Anal.Letters,* 32, 2725-2736, 1999.
27. Yu CJ, Wan, Y, Yowanto H, Li J, Tao C, James MD, Tan CL, Blackburn GF, Meade TJ. Electronic detection of single-base mismatches in DNA with ferrocene-modified probes. *J.Am.Chem.Soc,* 123, 11155-11161, 2001.
28. Erdem A, Ozsoz M. Electrochemical DNA biosensors based on DNA-drug interactions. *Electroanalysis* , 14 965- 974, 2002.
29. Palecek E, Fojta M. DNA hybridisation and damage, *Anal.Chem.* 2, 75 A-78A, 2001.
30. Gerard M, Chaubey A, Malhotra BD, Application of conducting polymers to biosensors, *Biosensors & Bioelectron.,* 17, 345-359, 2002.
31. Wang J. Electroanalysis and biosensors *Anal. Chem.,* 328-R-332R ,1999.