Nanotechnology-Based Cytogenetics

Cytogenetics has been used mainly to describe the chromosome structure and identify abnormalities related to disease. The localization of specific gene probes by fluorescent in situ hybridization (FISH)1 combined with conventional fluorescence microscopy has reached its limit. Molecular cytogenetics in now enhanced by use of nanobiotechnology, e.g., atomic force microscopy and quantum dot (QD) FISH.

Both atomic force microscopy and scanning near-field optical microscopy have been used to obtain local information from G-bands and chromosomal probes. The final resolution allows a more precise localization compared with standard techniques, and the extraction of very small amounts of chromosomal DNA by the scanning probe is possible. This method is also focused on the combination of biochemical and nanomanipulation techniques, which enable both nanodissection and nanoextraction of chromosomal DNA.

The photostability and narrow emission spectra of nonorganic QD fluorophores make them desirable candidates for the use of FISH to study the expression of specific mRNA transcripts. A method for direct QD labeling of modified oligonucleotide probes using streptavidin and biotin interactions increased sensitivity of multiple-label FISH (8). This technique also gives excellent histological results for FISH combined with immunohistochemistry.

QD's broad absorption spectra allowed different colored probes specific for distinct subnuclear genetic sequences to be simultaneously excited with a single excitation wavelength and imaged free of chromatic aberrations in a single exposure. A rapid method for the direct multicolor imaging of multiple subnuclear genetic sequences uses novel QD-based FISH. A Texas red dye gamma-satellite probe produces fluorescent foci at the periphery of interphase nucleus and labels every centromere in metaphase chromosomes (9).

Application of Nanoparticles for Tracking Stem Cells

A superparamagnetic iron oxide (SPIO) nanoparticle is emerging as an ideal probe for noninvasive cell tracking. However, its low intracellular labeling efficiency has limited its use and stimulated interest in the development of new labeling strategies.

The use of 200-nm perfluorocarbon nanoparticles to label endothelial progenitor cells taken from human umbilical cord blood enables in vivo progenitor cell detection by MRI. The MRI scanner can be tuned to the specific frequency of the fluorine compound in the nanoparticles,

and only the nanoparticle-containing cells are visible in the scan. This method eliminates any background signals, which often interfere with medical imaging. Moreover, the lack of interference enables measurement of very low amounts of the labeled cells and estimation of their number on the basis of the brightness of the image. Because several perfluorocarbon compounds are available, different types of cells could be labeled with different compounds, injected, and then detected separately by tuning the MRI scanner to the individual frequency of each cell type. This technology offers significant advantages over other cell-labeling technologies in development. Laboratory tests showed that the cells retained their usual surface markers and that they were still functional after the labeling process. The labeled cells were shown to migrate to and incorporate into blood vessels forming around tumors in mice. These methods could soon enable researchers and physicians to use unique signatures from the ingested nanoparticle beacons to directly track cells used in medical treatments. Such tracking ability could prove useful for monitoring tumors and diagnosing as well as treating cardiovascular problems.

Nanoscale Single-Cell or Molecule Identification

Nanotechnology has facilitated the development of methods for detection of single cells or a few molecules. Nanolaser scanning confocal spectroscopy, with the capability of single-cell resolution, can be used to identify previously unknown properties of certain cancer cells that distinguishes them from closely related nonpathogenic cells (11). Nanoproteomics, the application of nanobiotechnology to proteomics, can enable detection of a single molecule of protein (2). Biobarcode assays enable detection in body fluids of miniscule amounts of proteins that cannot be detected by conventional methods (12). A 2-dimensional method for mass spectrometry in solution is based on the interaction between a nanometer-scale pore and analytes (13). An applied electric current is used to force charged molecules (such as single-stranded DNA) one at a time into the nanopore, which is only 1.5 nm at its smallest point. As the molecules pass through the channel, the current flow is reduced in proportion to the size of each individual chain, allowing its mass to be easily derived. This single-molecule analysis technique could prove useful for the real-time characterization of biomarkers.

Application of Nanoparticles for Discovery of Biomarkers

Currently available molecular diagnostic technologies have been used to detect biomarkers of various diseases. Nanotechnology has refined the detection of biomarkers. Some biomarkers also form the basis of innovative molecular diagnostic tests. The physicochemical characteristics and high surface areas of nanoparticles make them ideal candidates for

developing biomarker-harvesting platforms. Given the variety of nanoparticle technologies that are available, it is feasible to tailor nanoparticle surfaces to selectively bind a subset of biomarkers and sequester them for later study using high-sensitivity proteomic tests (14). Biomarker harvesting is an underutilized application of nanoparticle technology and is likely to undergo substantial growth. Functional polymer-coated nanoparticles can be used for quick detection of biomarkers and DNA separation.

Nanoparticles for Molecular Diagnostics

Several nanoparticles have been used for diagnostics. Of these, the most frequently used are gold nanoparticles, QDs, and magnetic nanoparticles.

Gold Nanoparticles for Diagnostics

Small pieces of DNA can be attached to gold particles no larger than 13 nm in diameter. The gold nanoparticles assemble onto a sensor surface only in the presence of a complementary target. If a patterned sensor surface of multiple DNA strands is used, the technique can detect millions of different DNA sequences simultaneously.

The current nonoptimized detection limit of this method is 20 fmol/L. Gold nanoparticles are particularly good labels for sensors because a variety of analytical techniques can be used to detect them.

Quantum Dots

QDs are inorganic fluorophores that offer significant advantages over conventionally used fluorescent markers. They have high sensitivity, broad excitation spectra, stable fluorescence with simple excitation, and no need for lasers. Their red/infrared colors enable whole blood assays. QDs have a wide range of applications for molecular diagnostics and genotyping. QDs also enable multiplexed diagnostics and integration of diagnostics with therapeutics.

The most important potential applications of QDs are for cancer diagnosis. Luminescent and stable QD bioconjugates enable visualization of cancer cells in living animals. QDs can be combined with fluorescence microscopy to follow cells at high resolution in living animals. QDs have been coated with a polyacrylate cap and covalently linked to antibodies for immunofluorescent labeling of breast cancer marker Her2. Carbohydrate-encapsulated QDs with detectable luminescent properties are useful for imaging of cancer.

Another application of QDs is for viral diagnosis. Rapid and sensitive diagnosis of respiratory syncytial virus (RSV) is important for infection control and development of antiviral drugs. Antibody-conjugated nanoparticles rapidly and sensitively detect RSV and estimate relative

levels of surface protein expression (15). A major development is the use of dual-color QDs or fluorescence energy transfer nanobeads that can be simultaneously excited with a single light source. A QD system can detect the presence of particles of the RSV in a matter of hours. It is also more sensitive, allowing detection of the virus earlier in the course of an infection (16). When an RSV virus infects lung cells, it leaves part of its coat containing F and G proteins on the cell's surface. QDs have been linked to antibodies keyed to structures unique to the RSV coat. As a result, when QDs come in contact with either viral particles or infected cells they stick to their surface. In addition, colocalization of these viral proteins was shown using confocal microscopy.

Magnetic Nanoparticles

Iron nanoparticles, 15–20 nm in size and having saturation magnetization, have been synthesized and embedded in copolymer beads of styrene and glycidyl methacrylate (GMA), which were coated with polyGMA by seed polymerization (17). The resulting Fe/St-GMA/GMA beads had diameters of 100–200 nm. Coating with polyGMA changed the zeta potential of the beads from −93.7 to −54.8 mV, as measured by an electrophoresis method. As revealed by gel electrophoresis, this process facilitates nonspecific protein adsorption suppression, which is a requisite for nanoparticles to be applied to carriers for bioscreening.

Nanoparticles are used as labeling molecules for bioscreening. Superparamagnetic nanoparticles are useful for cell-tracking cells and for calcium sensing. Ferrofluids (Immunicon's CellTracks™ Technology) consist of a magnetic core surrounded by a polymeric layer coated with antibodies for capturing cells. A family of calcium indicators for MRI is formed by combining a powerful SPIO nanoparticle-based contrast mechanism with the versatile calcium-sensing protein calmodulin and its targets (18).

Superparamagnetic nanoparticles measuring 2–3 nm have been used in conjunction with MRI to reveal small and otherwise undetectable lymph-node metastases. Ultrasmall SPIO enhances MRI for imaging cerebral ischemic lesions. A dextran-coated iron oxide nanoparticle enhances MRI visualization of intracranial tumors for more than 24 h.

Safety Issues of Nanoparticles for Diagnostics

Potential toxic effects are a concern with in vivo use of nanoparticles but not with in vitro diagnostics, which forms the major portion of laboratory diagnostics. There are environmental concerns about the release of nanoparticles during manufacturing of nanoparticles and the environmental effects. These are being studied along with naturally present nanoparticles in the atmosphere.

There are still many unanswered questions about the fate of nanoparticles introduced into the living body. Because of the huge diversity of materials used and the wide range in size of nanoparticles, these effects will vary considerably. QDs made with fluorescent labels of calcium selenide or zinc sulfide to increase stability may release potentially toxic cadmium and zinc ions into cells. Capping QDs with ZnO effectively prevents Cd^{2+} formation on exposure to air but not to ultraviolet radiation, and the search for better coating materials is ongoing. A high-throughput gene expression test determined that specially coated QD fluorescent nanoprobes affect only 0.2% of the human genome, dispelling the concern that the mere presence of these potentially toxic sentinels disrupts cell function (19).

It is conceivable that particular sizes of some materials may have a bearing on toxic effects. A number of studies have been done, but at this stage, no conclusions can be drawn about the safety of nanoparticles. Concern centers around nanoparticles smaller than 20 nm in diameter, which can penetrate the cells. One limitation for the approval of in vivo nanomaterials for human diagnostics would be that demonstration of safety of nanoparticles would be required.

Nanobiosensors

Nanobiosensors are nanosensors used for detection of chemical or biological materials. Nanomaterials are exquisitely sensitive chemical and biological sensors (20). A classification of nanobiosensors is shown in Table 3

Table 3.

Nanobiosensors.

Electronic nanobiosensors
Electrochemical nanobiosensors
Ion channel switch biosensor technology
Nanowire biosensors
Cantilevers as biosensors
Carbon nanotube biosensors
FRET-based DNA nanosensor
Optical biosensors using laser, nanoshell, SPR, SERS, mRNA
PEBBLE (Probes Encapsulated by Biologically Localized Embedding)
Quartz nanobalance DNA sensor
Viral nanosensor

These sensors can be electronically gated to respond to the binding of a single molecule. Prototype sensors have demonstrated detection of nucleic acids, proteins, and ions. These sensors can operate in the liquid or gas phase, opening up an enormous variety of downstream applications. The detection schemes use inexpensive low-voltage measurement schemes and detect binding events directly, so there is no need for costly, complicated, and time-consuming labeling chemistries such as fluorescent dyes or the use of bulky and expensive optical detection systems. As a result, these sensors are inexpensive to manufacture and are portable. It may even be possible to develop implantable detection and monitoring devices on the basis of these detectors.

Clinical Applications of Nanodiagnostics

Some of the clinical applications of nanodiagnostics are mentioned along with technologies. This report briefly describes a few examples of the use of nanodiagnostics for diagnosis of cancer, infections, and neurological disorders. More detailed descriptions can be found in a handbook on nanomedicine (23).

Applications of Nanodiagnostics in Management of Cancer

Nanoparticles can be designed for dual-mode imaging of cancer. The best characteristics of QDs and magnetic iron oxide nanoparticles can be combined to create a single nanoparticle probe that can yield clinically useful images of both tumors and the molecules involved in cancer (24). Silica nanoparticles, approximately 30 nm in size, are impregnated with rhodamine, a bright fluorescent dye, and 9-nm diameter water-soluble iron oxide nanoparticles. The resulting combination of nanoparticles is approximately 45 nm in diameter and performs better in both MRI and fluorescent imaging tests than any of the individual components. An antibody that binds to polysialic acid molecules found on the surface of lung tumors is attached to these nanoparticles, which are quickly taken up by cultured tumor cells and can be visualized with fluorescence microscopy.

Bioconjugated QDs, collections of differently sized nanoparticles embedded in tiny polymer beads, provide a new class of biological labels for evaluating biomarkers on intact cells and tissue specimens. In particular, the use of multicolor QD probes in

immunohistochemistry is considered one of the most important and clinically relevant applications. The medical use of QD-based immunohistochemistry has been limited by the lack of specific instructions and protocols for clinicians. Preliminary results and detailed protocols for QD-antibody conjugation, tissue specimen preparation, multicolor QD staining, image processing, and biomarker quantification have been published (25). The results demonstrate that bioconjugated QDs can be used for multiplexed profiling of biomarkers, and ultimately for correlation with disease progression and response to therapy. These applications will increase the clinician's ability to predict the likely outcomes of drug therapy in a personalized approach to disease management. Bioinformatics and systems biology are used to link each patient's molecule profile with disease diagnosis and treatment decisions. The usefulness of these protocols was demonstrated by the simultaneous identification of multiple biomarkers in prostate cancer tissue. In general, QD bioconjugation is completed within 1 day, and multiplexed molecular profiling takes 1–3 days depending on the number of biomarkers and QD probes used.

Gold nanoparticles conjugated to anti–epidermal growth factor receptor monoclonal antibodies specifically and homogeneously bind to the surface of cancer cells with 600% greater affinity than to noncancerous cells (26). This specific and homogeneous binding is found to give a relatively sharper SPR absorption band with a red-shifted maximum compared with that observed when added to the noncancerous cells. Efficient conversion of strongly absorbed light by plasmonic gold nanoparticles to heat energy and their easy bioconjugation suggest their use as selective photothermal agents in molecular cancer cell targeting (27). Thus, gold nanoparticles can link diagnosis to therapeutics by noninvasively detecting the cancer and then destroying it.

Application of Nanodiagnostics in Infectious Diseases

The rapid and sensitive detection of pathogenic bacteria at the point of care is extremely important. Limitations of most of the conventional diagnostic methods are lack of ultrasensitivity and delay in getting results. A bioconjugated nanoparticle-based bioassay for in situ pathogen quantification can detect a single bacterium within 20-min (28). Detection of single-molecule hybridization has been achieved by a hybridization-detection method using multicolor oligonucleotide-functionalized QDs as nanoprobes (29). In the presence of various target sequences, combinatorial self-assembly of the nanoprobes via independent hybridization reactions leads to the generation of discernible sequence-specific spectral

codings. This method can be used for genetic analysis of anthrax pathogenicity by simultaneous detection of multiple relevant sequences.

A spectroscopic assay based on SERS using silver nanorods, which significantly amplify the signal, has been developed for rapid detection of trace levels of viruses with a high degree of sensitivity and specificity (30). The technique measures the change in frequency of a near-infrared laser as it scatters viral DNA or RNA. That change in frequency is as distinct as a fingerprint. This novel SERS assay can detect spectral differences between viruses, viral strains, and viruses with gene deletions in biological media. The method provides rapid diagnostics (≤60 s) for detection and characterization of viruses generating reproducible spectra without viral manipulation. This method is also inexpensive and easily reproducible.

Applications of Nanodiagnostics in Neurological Disorders

Nanoparticle contrast agents are in development to enhance MRI. A new MRI contrast agent using manganese oxide nanoparticles to visualize the anatomic structures of mouse brain produces images that are as clear as those obtained by histological examination. The new contrast agent will enable better research and diagnosis of neurological disorders such as Alzheimer disease, Parkinson disease, and stroke. Furthermore, antibodies can be attached to the manganese oxide nanoparticles, which recognize and specifically bind to receptors on the surface of breast cancer cells in mouse brains with breast cancer metastases. The tumors were clearly highlighted by the antibody-coupled contrast agent. The same principle should allow other disease-related changes or physiological systems to be visualized by using the appropriate antibodies.

Biosensors

The term "biosensor" is short for "biological sensor" and is a device made up of a transducer and a biological element that may be an enzyme, an antibody, or a nucleic acid. The biological element or bioelement interacts with the analyte being tested and the biological response is converted into an electrical signal by the transducer. Every biosensor has a biological component that acts as the sensor and an electronic component that detects and transmits the signal.

Biosensor = bioreceptor + transducer.

A biosensor consists of two components: a bioreceptor and a transducer. The bioreceptor is a biomolecule that recognizes the target analyte, and the transducer converts the recognition event into a measurable signal. The uniqueness of a biosensor is that the two components are integrated into one single sensor. This combination enables one to measure the target analyte without using reagents. For example, the glucose concentration in a blood sample can be measured directly by a biosensor made specifically for glucose measurement, by simply dipping the sensor in the sample. This is in contrast to the commonly performed assays, in which many sample preparation steps are necessary and each step may require a reagent to treat the sample. The simplicity and the speed of measurements that require no specialized laboratory skills are the main advantages of a biosensor.

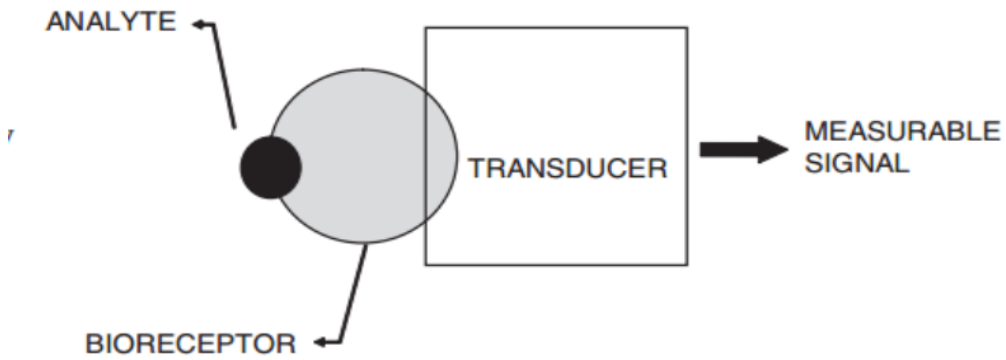


Figure 6.1.1: Biosensor configuration.

iosensor elements

A variety of substances may be used as the bioelement in a biosensor. Examples of these include:

- Nucleic acids
- Proteins including enzymes and antibodies. Antibody-based biosensors are also called immunosensors.
- Plant proteins or lectins
- Complex materials like tissue slices, microorganisms and organelles

The signal generated when the sensor interacts with the analyte may be electrical, optical or thermal. It is then converted by means of a suitable transducer into a measurable electrical parameter – usually a current or voltage.

Types of biosensors

Biosensors can be grouped according to the type of biological element and transducer they contain. They may also be named according to how the biosensing takes place.

The types of biological elements include:

- Enzymes
- Antibodies (also called immunosensors)
- Micro-organisms
- Biological tissue
- Organelles

Types of biosensing

The different ways that biosensing may occur are described below:

- If the bioelement binds to the analyte, the sensor is referred to as an affinity sensor.
- If the bioelement and the analyte give rise to a chemical change that can be used to measure the concentration of a substrate, the sensor is called a metabolic sensor.
- If the biological element combines with the analyte and does not change it chemically but converts it to an auxiliary substrate, the biosensor is called a catalytic sensor.

Types of sensing elements

Enzymes

An enzyme is a protein that has a high selectivity for a particular substrate, which it binds to, bringing about a catalytic change. Enzymes are commercially available in highly purified states and are therefore useful in the mass production of enzyme sensors. Enzymes can be fixed onto the surface of a transducer through adsorption, covalent attachment, and entrapment in a gel or an electrochemically generated polymer.

Antibodies or immunosensors

Antibodies are produced by B-lymphocytes in response to antigenic stimuli such as foreign invaders or microbes. When used as biosensors in immunoassays, antibodies are immobilized on the surface of a transducer through covalent attachment by conjugation of amino, carboxyl, aldehyde or sulfhydryl groups. Antibodies are sensitive to changes in pH, ionic strength, chemical inhibitors and temperature. Immune sensors usually employ optical, fluorescence or acoustic transducers.

Microorganisms

Microbes may be used to detect the consumption of oxygen or carbon dioxide in an environment using electrochemical techniques. Microbe biosensors have the advantage of

being cheaper than enzymes or antibodies and are more stable. However they may be less selective than enzymes or antibodies.

Other bioelements

Organelles, nucleic acids and biological tissues have been researched as biosensors.

Types of transducer

Electrochemical transducers

These are useful in electrochemical, amperometric and potentiometric signals. These electrodes are commonly made of platinum, gold, silver, stainless steel, or carbon-based inert materials.

Amperometric transducers, detect changes in current that occur due to oxidation or reduction. The current reflects the reaction that takes place between the analyte and the bioelement. Potentiometric transducers can measure the charge accumulation (potential) of an electrochemical cell. The transducer is usually made up of an ion-selective electrode and a reference electrode.

Optical transducers

Fluorescence is commonly used in signal transduction, especially when using enzymes and antibodies. Fibre optic probes consist of at least two fibres. One is connected to a light source of a given wavelength range and produces the excitation wave. The other is linked to the photodiode that detects the change in optical density at a selected wavelength. Plasmon resonance transducers measure alterations in the refractive index at and close to the sensing element's surface.

Acoustic transducers

These are devices in which mechanical acoustic waves act as the transduction system. The membrane contains chemically interactive materials in contact with a piezoelectric material. The devices vary according to the wave guiding process used. Usually, bulk acoustic wave (BAW) and surface acoustic wave (SAW) devices are used.

Calorimetric transduction

These measure the heat from the biochemical reaction between the sensing element and the analyte.

Surface Attachment of Biological Elements

A biosensor consists of a bioelement that interacts with an analyte and a transducer that converts the response into an electrical signal. The bioelement is usually an enzyme, antibody or microorganism and the transducer may be optical, acoustic, electrochemical or calorimetric.

The first step in preparing a biosensor is the application of the biological element to the surface of the sensor. The sensor may be made of a metal, a polymer or glass. The most common method for applying the bioelement is to coat the sensor with the biological element. The most commonly used bioelements include enzymes, antibodies, organelles, biological tissue and microbes. Coating of the sensor may be achieved using polylysine, aminosilane, epoxysilane or nitrocellulose to allow attachment to silicon chips or silica glass. Coating may also be achieved by fixing the bioelement on the surface layer by layer using alternatively charged polymer coatings. Sometimes, three dimensional lattices of hydrogel or xerogel are used chemically or physically to trap the bioelement on the surface. Chemical entrapment of the bioelements refers to strong chemical bonding that keeps the element in place, while physical entrapment means the element is unable to pass through the pores in the gel's matrix.

Sol-gel is the hydrogel that is usually employed and is a glassy silica created through the polymerization of silicate monomers in the presence of the biological elements using physical entrapment. Other hydrogels used include acrylate hydrogel, which polymerize upon radical initiation.

The bioelement and the sensor are coupled together in one of four ways:

- Membrane entrapment – A semipermeable membrane is used to separate the analyte and the bioelement. The sensor is attached to the bioelement.
- Physical adsorption – A combination of van der Waals forces, hydrophobic forces, hydrogen bonds, and ionic forces are used to attach the biomaterial to the sensor's surface.
- Matrix entrapment – Also called porous entrapment, a porous encapsulation matrix is created around the biological element to help it bind to the sensor.
- Covalent Bonding – The sensor surface is treated as a reactive group that the bioelement binds to.

Biosensor Characteristics.

Biosensors are characterized by eight parameters. These are:

(1) Sensitivity is the response of the sensor to per unit change in analyte concentration.

(2) Selectivity is the ability of the sensor to respond only to the target analyte. That is, lack of response to other interfering chemicals is the desired feature.

(3) Range is the concentration range over which the sensitivity of the sensor is good. Sometimes this is called dynamic range or linearity.

(4) Response time is the time required for the sensor to indicate 63% of its final response due to a step change in analyte concentration.
(5) Reproducibility is the accuracy with which the sensor's output can be obtained.
(6) Detection limit is the lowest concentration of the analyte to which there is a measurable response.
(7) Life time is the time period over which the sensor can be used without significant deterioration in performance characteristics.
(8) Stability characterizes the change in its baseline or sensitivity over a fixed period of time.

Considerations in Biosensor Development.

Once a target analyte has been identi- fied, the major tasks in developing a biosensor involve:
1. Selection of a suitable bioreceptor or a recognition molecule
2. Selection of a suitable immobilization method
3. Selection and design of a transducer that translates binding reaction into measurable signal
4. Design of biosensor considering measurement range, linearity, and minimization of interference, and enhancement of sensitivity
5. Packaging of the biosensor into a complete device

The first item above requires knowledge in biochemistry and biology, the second and third require knowledge in chemistry, electrochemistry and physics, and the fourth requires knowledge of kinetics and mass transfer. Once a biosensor has been designed, it must be packaged for convenient manufacturing and use. The current trend is miniaturization and mass production. Modern IC (integrated circuit) fabrication technology and micromachining technology are used increasingly in fabricating biosensors, as they reduce manufacturing costs. Therefore, an interdisciplinary research team, consisting of the various disciplines identified above, is essential for successful development of a biosensor.

Applications of Biosensor

Health Care

Measurement of Metabolites

The initial impetus for advancing sensor technology came from health care area, where it is now generally recognized that measurements of blood gases, ions and metabolites are often essential and allow a better estimation of the metabolic state of a patient. In intensive care units for example, patients frequently show rapid variations in biochemical

levels that require an urgent remedial action. Also, in less severe patient handling, more successful treatment can be achieved by obtaining instant assays. At present, the list of the most commonly required instant analyses is not extensive. In practice, these assays are performed by analytical laboratories, where discrete samples are analyzed, frequently using the more traditional analytical techniques.

Market Potential.

There is an increasing demand for inexpensive and reliable sensors to allow not only routine monitoring in the central or satellite laboratory, but also analysis with greater patient contact, such as in the hospital ward, emergency rooms, and operating rooms. Ultimately, patients themselves should be able to use biosensors in the monitoring and control of some treatable condition, such as diabetes. It is probably true to say that the major biosensor market may be found where an immediate assay is required. If the cost of laboratory maintenance are counted with the direct analytical costs, then low-cost biosensor devices can be desirable in the whole spectrum of analytical applications from hospital to home.

Diabetes.

The 'classic' and most widely explored example of closed-loop drugcontrol is probably to be found in the development of an artificial pancreas. Diabetic patients have a relative or absolute lack of insulin, a polypeptide hormone produced by the beta-cells of the pancreas, which is essential to the metabolism of a number of carbon sources. This deficiency causes various metabolic abnormalities, including higher than normal blood glucose levels. For such patients, insulin must be supplied externally. This has usually been achieved by subcutaneous injection, but fine control is difficult and hyperglycaemia cannot be totally avoided, or even hypoglycaemia is sometimes induced, causing impaired consciousness and the serious long-term complications to tissue associated with this intermittent low glucose condition.

Insulin Therapy.

Better methods for the treatment of insulin-dependent diabetes havebeen sought and infusion systems for continuous insulin delivery have been developed. However, regardless of the method of insulin therapy, its induction must be made in response to information on the current blood glucose levels in the patient. Three schemes are possible (Fig. 1.6), the first two dependent on discrete manual glucose measurement and the third a 'closed-loop' system, where insulin delivery is controlled by the output of a glucose sensor which is integrated with the insulin infuser. In the former case, glucose has been estimated on 'finger-prick' blood samples with a colorimetric test strip or more recently with an amperometric 'pen'-size

biosensor device by the patient themselves. Obviously these diagnostic kits must be easily portable, very simple to use and require the minimum of expert interpretation. However, even with the ability to monitor current glucose levels, intensive conventional insulin therapy requires multiple daily injections and is unable to anticipate future states between each application, where diet and exercise may require modification of the insulin dose. For example, it was shown that administration of glucose by subcutaneous injection, 60 min before a meal provides the best glucose/insulin management.

Artificial Pancreas.

The introduction of a closed-loop system, where integrated glucose measurements provide feedback control on a pre-programmed insulin administration based on habitual requirement, would therefore relieve the patient of frequent assay requirements and perhaps more desirably frequent injections. Ultimately, the closed-loop system becomes an artificial pancreas, where the glycaemic control is achieved through an implantable glucose sensor. Obviously, the requirements for this sensor are very different to those for the discrete measurement kits. As summarized in Table 1.4, the prolonged life-time and biocompatibility represent the major requirements.

Industrial Process Control

Bioreactor Control.

Real-time monitoring of carbon sources, dissolved gases,. in fermentation processes (Fig. 1.7a) could lead to optimization of the procedure giving increased yields at decreased materials cost. While real-time monitoring with feedback control involving automated systems does exist, currently only a few common variables are measured on-line (e.g. pH, temperature, CO2, O2)) which are often only indirectly related with the process under control.

Summary of potential applications for biosensors

- Clinical diagnosis and biomedicine
- Farm, garden and veterinary analysis
- Process control: fermentation control and analysis food and drink
- production and analysis
- Microbiology: bacterial and viral analysis
- Pharmaceutical and drug analysis
- Industrial effluent control

- Pollution control and monitoring o Mining, industrial and toxic gases
- Military applications

MICROFLUIDICS

Microfluidics is a multidisciplinary field intersecting engineering, physics, chemistry, biochemistry, nanotechnology, and biotechnology, with practical applications to the design of systems in which low volumes of fluids are processed to achieve multiplexing, automation, and high-throughput screening. Microfluidics emerged in the beginning of the 1980s and is used in the development of inkjet printheads, DNA chips, lab-on-a-chip technology, micro-propulsion, and micro-thermal technologies. It deals with the behavior, precise control and manipulation of fluids that are geometrically constrained to a small, typically sub-millimeter, scale. Typically, **micro** means one of the following features:

- small volumes (µL, nL, pL, fL)
- small size
- low energy consumption
- effects of the micro domain

Typically fluids are moved, mixed, separated or otherwise processed. Numerous applications employ passive fluid control techniques like capillary forces. In some applications external actuation means are additionally used for a directed transport of the media. Examples are rotary drives applying centrifugal forces for the fluid transport on the passive chips. **Active microfluidics** refers to the defined manipulation of the working fluid by active (micro) components such as micropumps or micro valves. Micro pumps supply fluids in a continuous manner or are used for dosing. Micro valves determine the flow direction or the mode of movement of pumped liquids. Often processes which are normally carried out in a lab are miniaturized on a single chip in order to enhance efficiency and mobility as well as reducing sample and reagent volumes.

Microscale behavior of fluids

The behavior of fluids at the microscale can differ from 'macrofluidic' behavior in that factors such as surface tension, energy dissipation, and fluidic resistance start to dominate the system. Microfluidics studies how these behaviors change, and how they can be worked around, or exploited for new uses. At small scales (channel diameters of around 100 nanometers to several hundred micrometers) some interesting and sometimes unintuitive

properties appear. In particular, the Reynolds number (which compares the effect of momentum of a fluid to the effect ofviscosity) can become very low. A key consequence of this is that fluids, when side-by-side, do not necessarily mix in the traditional sense, as flow becomes laminar rather than turbulent; molecular transport between them must often be through diffusion. igh specificity of chemical and physical properties (concentration, pH, temperature, shear force, etc.) can also be ensured resulting in more uniform reaction conditions and higher grade products in single and multi-step reactions

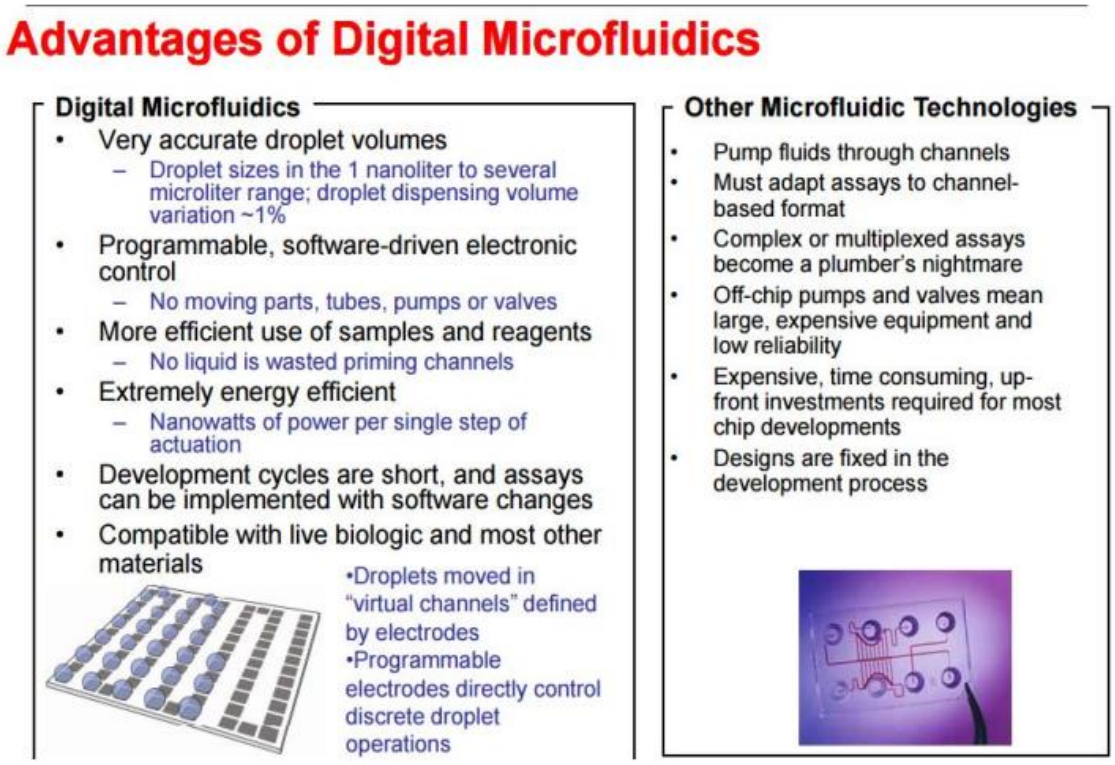
Microfluidics-based biochips have become an actively researched area in recent years. Sometimes also referred to as lab-on-a-chip, biochips integrate different biochemical analysis functionalities (e.g., dispensers, filters, mixers, separators, detectors) on-chip, miniaturizing the macroscopic chemical and biological processes to a sub-millimetre scale [1]. These microsystems offer several advantages over the conventional biochemical analyzers, e.g., reduced sample and reagent volumes, speeded up biochemical reactions, ultra-sensitive detection and higher system throughput, with several assays being integrated on the same chip

The key component of continuous-flow biochips is an onchip micromechanical valve, which is analogous to a transistor in microelectronics. The biochip has two logical layers: flow layer and the control layer. The liquid in the flow layer is manipulated using the control layer. A valve is formed at the cross section of channels in corresponding layers. Typically, micromechanical valves are made of silicone rubber (polydimethylsiloxane, PDMS) and actuated by applying fluidic pressure to the elastomeric membrane. The external pneumatic air pressure that is applied to the membrane is controlled using a solenoid valve

Microfluidic-based biochips are soon revolutionizing clinical diagnostics and many biochemical laboratory procedures due to their advantages of automation, cost reduction, portability, and efficiency [18]. Conventional technology depends on the manipulation of continuous liquid flow through microfabricated channels. However, actuation of flow is implemented with external assistance of micro-pump and micro-valve, which are complex and cumbersome. Moreover, permanently-etched channels greatly restrict the feasibility and versatility. Regarding this, microfluidic research is witnessing a paradigm shift from the continuous-flow-based architecture to droplet-based architecture or, in particular, the so-called digital microfluidic biochip (DMFB)

Generally, a DMFB consists of a two-dimensional (2D) electrode array and peripheral devices (e.g., optical detector, dispensing port, etc.), as schematically shown in Figure 1(a) [11, 18]. The sample carriers on DMFBs, droplets, being miniaturized and discretized liquids,

are controlled by underlying electrodes using electrical actuations (i.e., a principle called electrowetting-on-dielectric or EWOD) [15]. By assigning time-varying voltage values to turn on/off electrodes, droplets can be moved around the entire 2D array to perform fundamental operations (e.g., dispensing and mixing) [7, 16]. These operations are carried out in a reconfigurable manner due to their flexibility in area and time domain [4]. Compared with continuous- flow-based biochips, DMFBs offer various advantages including more flexible control mechanism and higher throughput and sensitivity as well as lower sample/reagent volume consumption. Due to these advantages, DMFBs have attracted much effort devoted to serving the need from marketplace such as healthcare, environmental, and toxin monitoring applications. As reported in Figure 1(b) [2], the global market value for biochip products is an estimated $2.6 billion in 2009, but is expected to increase to nearly $6 billion in 2014, for a 5- year high compound-annual-growth-rate (CAGR) of 17.7%. Continuing growth of various applications have dramatically complicated the chip/system integration and design complexity [5, 11], making traditional manual designs not suitable enough especially under the time-to-market circumstance.



Applications of Digital Microfluidic Biochips

- Drug discovery and biotechnology
  - Proteomics
  - High-throughput screening
  - Genomics
- Medical diagnostics and therapeutics
  - Clinical chemistry
  - Immunoassays
  - Nucleic acid tests
- Environmental and other applications
  - Micro-optics
  - Countering bioterrorism
  - Air/water/agro food monitoring