Bio-MEMS FABRICXATION LABORATORY

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CHAPTER 1: INTRODUCTION

1.1. Definition

Bio-MEMS is an abbreviation for biomedical (or biological) microelectromechanical systems. Bio-MEMS have considerable overlap, and are sometimes considered synonymous, with lab-on-a-chip (LOC) and micro total analysis systems (μTAS). Bio-MEMS are typically more focused on mechanical parts and microfabrication technologies made suitable for biological applications. On the other hand, lab-on-a-chip is concerned with miniaturization and integration of laboratory processes and experiments into single (often microfluidic) chips. In this definition, lab-on-a-chip devices do not strictly have biological applications, although most do or are amendable to be adapted for biological purposes. Similarly, micro total analysis systems may not have biological applications in mind, and are usually dedicated to chemical analysis. A broad definition for bio-MEMS can be used to refer to the science and technology of operating at the microscale for biological and biomedical applications, which may or may not include any electronic or mechanical functions. The interdisciplinary nature of bio-MEMS combines material sciences, clinical sciences, medicine, surgery, electrical engineering, mechanical engineering, optical engineering, chemical engineering, and biomedical engineering. Some of its major applications include genomics, proteomics, molecular diagnostics, point-of-care diagnostics, tissue engineering, single cell analysis and implantable microdevices.
A Venn diagram outlining and contrasting some aspects of the fields of bio-MEMS, lab-on-a-chip, μTAS

1.2. History

In 1967, S. B. Carter reported the use of shadow-evaporated palladium islands for cell attachment. After this first bio-MEMS study, subsequent development in the field was slow for around 20 years. In 1985, Unipath Inc. commercialized ClearBlue, a pregnancy test still used today that can be considered the first microfluidic device containing paper and the first microfluidic product to market. In 1990, Andreas Manz and H. Michael Widmer from Ciba-Geigy (now Novartis), Switzerland first coined the term micro total analysis system (μTAS) in their seminal paper proposing the use of miniaturized total chemical analysis systems for chemical sensing. There have been three major motivating factors behind the concept of μTAS. Firstly, drug discovery in the last decades leading up to the 1990s had been limited due to the time and cost of running many chromatographic analyses in parallel on macroscopic equipment. Secondly, the Human Genome Project (HGP), which started in October 1990, created demand for improvements in DNA sequencing capacity. Capillary electrophoresis thus became a focus for chemical and DNA separation.

Thirdly, DARPA of the US Department of Defense supported a series of microfluidic research programs in the 1990s after realizing there was a need to develop field-deployable microsystems for the detection of chemical and biological agents that were potential military and terrorist threats. Researchers started to use photolithography equipment for microfabrication of microelectromechanical systems (MEMS) as inherited from the microelectronics industry. At the time, the application of MEMS to biology was limited because this technology was optimized for silicon or glass wafers and used solvent-based photoresists that were not compatible with biological material. In 1993, George M. Whitesides, a Harvard chemist, introduced inexpensive PDMS-based microfabrication and this revolutionized the bio-MEMS field. Since then, the field of bio-MEMS has exploded. Selected major technical achievements during bio-MEMS development of the 1990s include:

- In 1991, the first oligonucleotide chip was developed
In 1998, the first solid microneedles were developed for drug delivery
In 1998, the first continuous-flow polymerase chain reaction chip was developed
In 1999, the first demonstration of heterogeneous laminar flows for selective treatment of cells in microchannels

Today, hydrogels such as agarose, biocompatible photoresists, and self-assembly are key areas of research in improving bio-MEMS as replacements or complements to PDMS.

1.3. Approaches

1.3.1. Materials

1.3.1.1. Silicon and glass

Conventional micromachining techniques such as wet etching, dry etching, deep reactive ion etching, sputtering, anodic bonding, and fusion bonding have been used in bio-MEMS to make flow channels, flow sensors, chemical detectors, separation capillaries, mixers, filters, pumps and valves. However, there are some drawbacks to using silicon-based devices in biomedical applications such as their high cost and bioincompatibility. Due to being single-use only, larger than their MEMS counterparts, and the requirement of clean room facilities, high material and processing costs make silicon-based bio-MEMS less economically attractive. In vivo, silicon-based bio-MEMS can be readily functionalized to minimize protein adsorption, but the brittleness of silicon remains a major issue.

1.3.1.2. Plastics and polymers

Using plastics and polymers in bio-MEMS is attractive because they can be easily fabricated, compatible with micromachining and rapid prototyping methods, as well as have low cost. Many polymers are also optically transparent and can be integrated into systems that use optical detection techniques such as fluorescence, UV/Vis absorbance, or Raman method. Moreover, many polymers are biologically compatible, chemically inert to solvents, and electrically insulating for applications where strong electrical fields are necessary such as electrophoretic
separation. Surface chemistry of polymers can also be modified for specific applications. The most common polymers used in bio-MEMS include PMMA, PDMS, OSTEmer and SU-8.

A) Micropatterning of fibronectin on PNIPAM glass surface. B) and C) Single fibroblasts are spatially constrained to the geometry of the fibronectin micropattern.

1.3.1.3. Biological materials

Microscale manipulation and patterning of biological materials such as proteins, cells and tissues have been used in the development of cell-based arrays, microarrays, microfabrication based tissue engineering, and artificial organs. Biological micropatterning can be used for high-throughput single cell analysis, precise control of cellular microenvironment, as well as controlled integration of cells into appropriate multi-cellular architectures to recapitulate in vivo conditions. Photolithography, microcontact printing, selective microfluidic delivery, and self-assembled monolayers are some methods used to pattern biological molecules onto surfaces. Cell micropatterning can be done using microcontact patterning of extracellular matrix proteins, cellular electrophoresis, optical tweezer arrays, dielectrophoresis, and electrochemically active surfaces.

1.3.1.4. Paper

Paper microfluidics (sometimes called lab on paper) is the use of paper substrates in microfabrication to manipulate fluid flow for different applications. Paper microfluidics have been applied in paper electrophoresis and immunoassays, the most notable being the commercialized pregnancy test, ClearBlue. Advantages of using paper for microfluidics and
electrophoresis in bio-MEMS include its low cost, biodegradability, and natural wicking action. Compared to traditional microfluidic channels, paper microchannels are accessible for sample introduction (especially forensic-style samples such as body fluids and soil), as well as its natural filtering properties that exclude cell debris, dirt, and other impurities in samples. Techniques for micropatterning paper include photolithography, laser cutting, ink jet printing, plasma treatment, and wax patterning.

i. Motivation

Paper-based microfluidic devices are devices that can control and manipulate small amount of liquid on the cellulose substrate. Cellulose, as a major component of all plant matter, is one of the most abundant biopolymers in nature. Compared to traditional microfluidics, cellulose substrates provide the opportunity for applications to be low-cost, low weight, and flexible. Moreover, fluid flow can be easily driven by capillary force within the microchannel, which requires no external power supply. The porous structure of cellulose provides for a high-surface area to volume ratio, which can increase the sensor performance of these types of devices. Cellulose substrates also have ability to store reagents in active form within the channel. All these advantages of using cellulose, makes it as an ideal material for microfluidics devices.

ii. Challenges

Compared to traditional microfluidics, paper-based microfluidics still has the following limitations: (1) The small sample retention, (2) For sample with low surface tension, some hydrophobic agents for patterning devices are not strong enough, (3) The limit of detection is usually high for the colorimetric detection method.

iii. Fabrication methods

Cellulose paper is a hydrophilic substrate. In order to fabricate microfluidics channel, specific methods need to be used to tune the hydrophobicity of the cellulose substrate. Two mechanisms were applied to pattern microfluidics channels. One is to selectively fabricate a hydrophobic surface onto cellulose film. Another is used to modify cellulose film as a hydrophobic substrate first, and then, selectively tune part of surface back to hydrophilic surface. Until now, different
techniques have been proved to be feasible to create microfluidics channels on the cellulose substrate, such as wax printing, inkjet printing, photolithography, flexographic printing, screen printing, laser cutting and plasma treatment. Researchers have divided these methods into four different categories.

a. Wax Printing

Wax printing is a technique to use wax as a hydrophobic agent on the cellulose substrate. Wax can be easily printed by wax pen, inkjet printer or wax printer onto the surface of cellulose membrane. And then, the wax can be melted, allowing it to penetrate through the cellulose under mild heat treatment. The surface with wax turns to a hydrophobic surface. Wax printing is a simple, rapid, and environmentally-friendly method for patterning. The disadvantage of this method is that researchers need expensive wax printer and extra heating process to complete the process.

b. Inkjet Printing

Inkjet printing is a new type of computer printing method to transfer digital design onto different substrates with droplets of ink. Specific biomolecules can be precisely printed onto the sensing zone of the paper-based microfluidics device. As for patterning microfluidics channel, inkjet etching and inkjet printing are developed. For inkjet etching, toluene was used as inkjet agent to selectively remove hydrophobic polystyrene that was prepatterned on the paper. For inkjet printing, hydrophobic agents, such as alkyl ketene dimer, were directly printed on the paper to form hydrophobic barrier. The advantage of inkjet printing is high resolution, wide choice of inks. Electrodes can also be printed by this method. The disadvantage of this process is that the speed of printing is not feasible for massive production, unless roll-to-roll fabrication is used.

c. Photolithography

Photoresist-saturated paper is exposed to UV light through a photomask. After exposure, uncured photoresist can be removed by organic solvent. Cured photoresist form as a hydrophobic barrier on the paper. The fabrication process is rapid and allows for high resolution. The drawback is that photolithography needs the use of organic solvents and expensive photoresists.


d. Flexographic printing

Flexographic printing is a high throughput fabrication technique. Olkkonen et al. used flexographic printing with polystyrene ink to fabricate microfluidics channel on paper substrate. This method can be easily scale-up, the speed of printing can be greater than 300 m/min. The disadvantage of it is that different printing plates are needed. Meanwhile, it can only print one reagent at one time.

e. Screen printing

Screen printing is a printing method that use a mesh to transfer ink on the substrate, unless the area that was protected by blocking stencil. The process of screen printing is simple; however, the resolution is low and different blocking stencils are needed for different designs.

f. Laser Cutting

The laser cutting used computer to control CO$_2$ laser to cut paper substrate into specific design. No chemicals are needed during process. The disadvantage of this method is expense of laser cutter is far more than a knife and the power of laser is strong that cause paper substrate warping or tearing.

g. Plasma treatment

Cellulose substrate was pre-modified via octadecyltrichlorosilane silanization to fabricate a hydrophobic surface. Plasma treatment with a mask will let exposed area of the substrate turn back to hydrophilic; due to degradation of octadecyltrichlorosilane. The drawback of this method is that the substrate under a mask is easily over-etched.

iv. Application

The main application for paper-based microfluidics is to provide a low-cost, user-friendly analytic platform for assay diagnosis. Pregnant test is one of well-known products for paper-based microfluidics. Researchers have used paper-based microfluidics for a wide range of applications, such as biochemical detection, immunological detection and molecular detection.
a. Biochemical detection

Many analytes have been proved that they can be detected with paper-based microfluidics. In the detection zone on the paper substrate, the analytes can have a chemical reaction with the immobilized reagent and develop a signal. The signal can be detected by different methods, such as colorimetric, electrochemical, fluorescent, chemiluminiscence (CL). Lopez-Ruiz et al. successfully developed paper microfluidic devices with applications of pH and nitrite colorimetric determination.

b. Immunological detection

Immunological detection is to detect analytes with immunoassay technique. Antibody or protein can be covalently bonded on the paper substrate with surface modification. One of advantages for paper-based microfluidics in immunological detection is that paper substrate have capability of storage reagent in active form. It is crucial for applications in low resource setting and point of care application.

c. Molecular detection
Sequence-specific detection of nucleic acid hybridization has been proved that they can be detected with paper-based microfluidics. The sequence with a tag or change in the concentration can be targeted by capture probe, letting the reaction visible or measurable.

1.4. Electrokinetics

An electrophoresis experiment example: Two conical electrodes are set at both the inlet and outlet of a microchannel and cells are moved along the microchannel by an applied DC electric field. Electrokinetics has been exploited in bio-MEMS for separating mixtures of molecules and cells using electrical fields. In electrophoresis, a charged species in a liquid moves under the influence of an applied electric field. Electrophoresis has been used to fractionate small ions, charged organic molecules, proteins, and DNA. Electrophoresis and microfluidics are highly synergistic because it is possible to use higher voltages in microchannels due to faster heat removal. Isoelectric focusing is the separation of proteins, organelles, and cells with different isoelectric points. Isoelectric focusing requires a pH gradient (usually generated with electrodes) perpendicular to the flow direction. Sorting and focusing of the species of interest is achieved because an electrophoretic force causes perpendicular migration until it flows along its respective isoelectric points. Dielectrophoresis is the motion of uncharged particles due to induced polarization from nonuniform electric fields. Dielectrophoresis can be used in bio-MEMS for dielectrophoresis traps, concentrating specific particles at specific points on surfaces, and diverting particles from one flow stream to another for dynamic concentration. For specific types of electrophoresis (for example, the process of administering medicine, iontophoresis), see Electrophoresis (disambiguation).
Electrophoresis is the motion of dispersed particles relative to a fluid under the influence of a spatially uniform electric field. This electrokinetic phenomenon was observed for the first time in 1807 by Russian professors Peter Ivanovich Strakhov and Ferdinand Frederic Reuss (Moscow State University), who noticed that the application of a constant electric field caused clay particles dispersed in water to migrate. It is ultimately caused by the presence of a charged interface between the particle surface and the surrounding fluid. It is the basis for a number of analytical techniques used in chemistry for separating molecules by size, charge, or binding affinity. Electrophoresis of positively charged particles (cations) is called cataphoresis, while electrophoresis of negatively charged particles (anions) is called anaphoresis. Electrophoresis is a technique used in laboratories in order to separate macromolecules based on size. The
technique applies a negative charge so proteins move towards a positive charge. This is used for both DNA and RNA analysis. Polyacrylamide gel electrophoresis (PAGE) has a clearer resolution than agarose and is more suitable for quantitative analysis. In this technique DNA footprinting can identify how proteins bind to DNA. It can be used to separate proteins by size, density and purity. It can also be used for plasmid analysis, which develops our understanding of bacteria becoming resistant to antibiotics.

1.4.1. Theory

Suspended particles have an electric surface charge, strongly affected by surface adsorbed species, on which an external electric field exerts an electrostatic Coulomb force. According to the double layer theory, all surface charges in fluids are screened by a diffuse layer of ions, which has the same absolute charge but opposite sign with respect to that of the surface charge. The electric field also exerts a force on the ions in the diffuse layer which has direction opposite to that acting on the surface charge. This latter force is not actually applied to the particle, but to the ions in the diffuse layer located at some distance from the particle surface, and part of it is transferred all the way to the particle surface through viscous stress. This part of the force is also called electrophoretic retardation force. When the electric field is applied and the charged particle to be analyzed is at steady movement through the diffuse layer, the total resulting force is zero: Considering the drag on the moving particles due to the viscosity of the dispersant, in the case of low Reynolds number and moderate electric field strength $E$, the drift velocity of a dispersed particle $v$ is simply proportional to the applied field, which leaves the electrophoretic mobility $\mu_e$ defined as:

The most well known and widely used theory of electrophoresis was developed in 1903 by Smoluchowski: where $\varepsilon_r$ is the dielectric constant of the dispersion medium, $\varepsilon_0$ is the permittivity of free space $(C^2 N^{-1} m^{-2})$, $\eta$ is dynamic viscosity of the dispersion medium (Pa s), and $\zeta$ is zeta potential (i.e., the electrokinetic potential of the slipping plane in the double layer). The Smoluchowski theory is very powerful because it works for dispersed particles of any shape at any concentration. It has limitations on its validity. It follows, for instance, because it does not include Debye length $\kappa^{-1}$. However, Debye length must be important for electrophoresis.
Increasing thickness of the double layer (DL) leads to removing the point of retardation force further from the particle surface. The thicker the DL, the smaller the retardation force must be.

Detailed theoretical analysis proved that the Smoluchowski theory is valid only for sufficiently thin DL, when particle radius $a$ is much greater than the Debye length: This model of "thin double layer" offers tremendous simplifications not only for electrophoresis theory but for many other electrokinetic theories. This model is valid for most aqueous systems, where the Debye length is usually only a few nanometers. It only breaks for nano-colloids in solution with ionic strength close to water. The Smoluchowski theory also neglects the contributions from surface conductivity. In the effort of expanding the range of validity of electrophoretic theories, the opposite asymptotic case was considered, when Debye length is larger than particle radius. Under this condition of a "thick double layer", Hückel predicted the following relation for electrophoretic mobility: This model can be useful for some nanoparticles and non-polar fluids, where Debye length is much larger than in the usual cases. There are several analytical theories that incorporate surface conductivity and eliminate the restriction of a small Dukhin number, pioneered by Overbeek. and Booth. Modern, rigorous theories valid for any Zeta potential and often any $a\kappa$ stem mostly from Dukhin–Semenikhin theory. In the thin double layer limit, these theories confirm the numerical solution to the problem provided by O'Brien and White.

1.5. Microfluidics

When multiple solutions are added into the same microchannel, they flow in separate flow lanes (no mixing) due to laminar flow characteristics. Microfluidics refers to systems that manipulate small ($\mu$L, nL, pL, fL) amounts of fluids on microfabricated substrates. Microfluidic approaches to bio-MEMS confer several advantages:
- Flow in microchannels is laminar, which allows selective treatment of cells in microchannels, mathematical modelling of flow patterns and concentrations, as well as quantitative predictions of the biological environment of cells and biochemical reactions
- Microfluidic features can be fabricated on the cellular scale or smaller, which enables investigation of (sub)cellular phenomena, seeding and sorting of single cells, and recapitulation of physiological parameters
- Integration of microelectronics, micromechanics, and microoptics onto the same platform allows automated device control, which reduces human error and operation costs
- Microfluidic technology is relatively economical due to batch fabrication and high-throughput (parallelization and redundancy). This allows the production of disposable or single-use chips for improved ease of use and reduced probability of biological cross contamination, as well as rapid prototyping
- Microfluidic devices consume much smaller amounts of reagents, can be made to require only a small amount of analytes for chemical detection, require less time for processes and reactions to complete, and produces less waste than conventional macrofluidic devices and experiments
- Appropriate packaging of microfluidic devices can make them suitable for wearable applications, implants, and portable applications in developing countries

An interesting approach combining electrokinetic phenomena and microfluidics is digital microfluidics. In digital microfluidics, a substrate surface is micropatterned with electrodes and selectively activated. Manipulation of small fluid droplets occurs via electrowetting, which is the phenomenon where an electric field changes the wettability of an electrolyte droplet on a surface.

Microfluidics deals with the behaviour, precise control and manipulation of fluids that are geometrically constrained to a small, typically sub-millimeter, scale. It is a multidisciplinary field at the intersection of engineering, physics, chemistry, biochemistry, nanotechnology, and biotechnology, with practical applications in 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.
Typically, **micro** means one of the following features:

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

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 microvalves. Micropumps supply fluids in a continuous manner or are used for dosing. Microvalves determine the flow direction or the mode of movement of pumped liquids. Often processes which are normally carried out in a lab are miniaturised on a single chip in order to enhance efficiency and mobility as well as reducing sample and reagent volumes.

1.5.1. *Microscale behavior of fluids*

The behaviour of fluids at the microscale can differ from "macrofluidic" behaviour in that factors such as surface tension, energy dissipation, and fluidic resistance start to dominate the system. Microfluidics studies how these behaviours change, and how they can be worked around, or exploited for new uses. At small scales (channel size of around 100 nanometers to 500 micrometers) some interesting and sometimes unintuitive properties appear. In particular, the Reynolds number (which compares the effect of the momentum of a fluid to the effect of viscosity) can become very low. A key consequence is co-flowing fluids do not necessarily mix in the traditional sense, as flow becomes laminar rather than turbulent; molecular transport between them must often be through diffusion. High 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.

1.5.2. Key application areas

Microfluidic structures include micropneumatic systems, i.e. microsystems for the handling of off-chip fluids (liquid pumps, gas valves, etc.), and microfluidic structures for the on-chip handling of nanoliter (nl) and picoliter (pl) volumes. To date, the most successful commercial application of microfluidics is the inkjet printhead. Additionally, advances in microfluidic manufacturing allow the devices to be produced in low-cost plastics and part quality may be verified automatically.
Microfluidic synthesis of functionalized quantum dots for bioimaging

Advances in microfluidics technology are revolutionizing molecular biology procedures for enzymatic analysis (e.g., glucose and lactate assays), DNA analysis (e.g., polymerase chain reaction and high-throughput sequencing), and proteomics. The basic idea of microfluidic biochips is to integrate assay operations such as detection, as well as sample pre-treatment and sample preparation on one chip. An emerging application area for biochips is clinical pathology, especially the immediate point-of-care diagnosis of diseases. In addition, microfluidics-based devices, capable of continuous sampling and real-time testing of air/water samples for biochemical toxins and other dangerous pathogens, can serve as an always-on "bio-smoke alarm" for early warning. Microfluidic technology has led to the creation of powerful tools for biologists to control the complete cellular environment, leading to new questions and discoveries.

Many diverse advantages of this technology for microbiology are listed below:

- General single cell studies including growth
- Cellular aging: microfluidic devices such as the "mother machine" allow tracking of thousands of individual cells for many generations until they die.
- Microenvironmental control: ranging from mechanical environment to chemical environment
- Precise spatiotemporal concentration gradients by incorporating multiple chemical inputs to a single device
- Force measurements of adherent cells or confined chromosomes: objects trapped in a microfluidic device can be directly manipulated using optical tweezers or other force-generating methods
- Confining cells and exerting controlled forces by coupling with external force-generation methods such as Stokes flow, optical tweezer, or controlled deformation of the PDMS device
- Fast and precise temperature control
- Electric field integration
- Plant on a chip and plant tissue culture
• Antibiotic resistance: microfluidic devices can be used as heterogeneous environments for microorganisms. In a heterogeneous environment, it is easier for a microorganism to evolve. This can be useful for testing the acceleration of evolution of a microorganism / for testing the development of antibiotic resistance.

Some of these areas are further elaborated in the sections below.

1.5.2.1. Continuous-flow microfluidics

These technologies are based on the manipulation of continuous liquid flow through microfabricated channels. Actuation of liquid flow is implemented either by external pressure sources, external mechanical pumps, integrated mechanical micropumps, or by combinations of capillary forces and electrokinetic mechanisms. Continuous-flow microfluidic operation is the mainstream approach because it is easy to implement and less sensitive to protein fouling problems. Continuous-flow devices are adequate for many well-defined and simple biochemical applications, and for certain tasks such as chemical separation, but they are less suitable for tasks requiring a high degree of flexibility or fluid manipulations. These closed-channel systems are inherently difficult to integrate and scale because the parameters that govern flow field vary along the flow path making the fluid flow at any one location dependent on the properties of the entire system. Permanently etched microstructures also lead to limited reconfigurability and poor fault tolerance capability. Process monitoring capabilities in continuous-flow systems can be achieved with highly sensitive microfluidic flow sensors based on MEMS technology which offers resolutions down to the nanoliter range.

1.5.2.2. Droplet-based microfluidics

Droplet-based microfluidics is a subcategory of microfluidics in contrast with continuous microfluidics; droplet-based microfluidics manipulates discrete volumes of fluids in immiscible phases with low Reynolds number and laminar flow regimes. Interest in droplet-based microfluidics systems has been growing substantially in past decades. Microdroplets allow for handling miniature volumes (μl to fl) of fluids conveniently, provide better mixing, encapsulation, sorting, and sensing, and suit high throughput experiments. Exploiting the benefits of droplet-based microfluidics efficiently requires a deep understanding of droplet
generation to perform various logical operations such as droplet motion, droplet sorting, droplet merging, and droplet breakup.

1.5.2.3. Digital microfluidics

Alternatives to the above closed-channel continuous-flow systems include novel open structures, where discrete, independently controllable droplets are manipulated on a substrate using electrowetting. Following the analogy of digital microelectronics, this approach is referred to as digital microfluidics. Le Pesant et al. pioneered the use of electrocapillary forces to move droplets on a digital track. The "fluid transistor" pioneered by Cytonix also played a role. The technology was subsequently commercialised by Duke University. By using discrete unit-volume droplets, a microfluidic function can be reduced to a set of repeated basic operations, i.e., moving one unit of fluid over one unit of distance. This "digitisation" method facilitates the use of a hierarchical and cell-based approach for microfluidic biochip design. Therefore, digital microfluidics offers a flexible and scalable system architecture as well as high fault-tolerance capability.

Moreover, because each droplet can be controlled independently, these systems also have dynamic reconfigurability, whereby groups of unit cells in a microfluidic array can be reconfigured to change their functionality during the concurrent execution of a set of bioassays. Although droplets are manipulated in confined microfluidic channels, since the control on droplets is not independent, it should not be confused as "digital microfluidics". One common actuation method for digital microfluidics is electrowetting-on-dielectric (EWOD). Many lab-on-a-chip applications have been demonstrated within the digital microfluidics paradigm using electrowetting. However, recently other techniques for droplet manipulation have also been demonstrated using surface acoustic waves, optoelectrowetting, mechanical actuation, etc.

1.5.2.4. DNA chips (microarrays)

Early biochips were based on the idea of a DNA microarray, e.g., the Gene Chip DNA array from Affymetrix, which is a piece of glass, plastic or silicon substrate, on which pieces of DNA (probes) are affixed in a microscopic array. Similar to a DNA microarray, a protein array is a miniature array where a multitude of different capture agents, most frequently monoclonal
antibodies, are deposited on a chip surface; they are used to determine the presence and/or amount of proteins in biological samples, e.g., blood. A drawback of DNA and protein arrays is that they are neither reconfigurable nor scalable after manufacture. Digital microfluidics has been described as a means for carrying out Digital PCR.

1.5.2.5. Molecular biology

In addition to microarrays, biochips have been designed for two-dimensional electrophoresis, transcriptome analysis, and PCR amplification. Other applications include various electrophoresis and liquid chromatography applications for proteins and DNA, cell separation, in particular, blood cell separation, protein analysis, cell manipulation and analysis including cell viability analysis and microorganism capturing.

1.5.2.6. Evolutionary biology

By combining microfluidics with landscape ecology and nanofluidics, a nano/micro fabricated fluidic landscape can be constructed by building local patches of bacterial habitat and connecting them by dispersal corridors. The resulting landscapes can be used as physical implementations of an adaptive landscape, by generating a spatial mosaic of patches of opportunity distributed in space and time. The patchy nature of these fluidic landscapes allows for the study of adapting bacterial cells in a metapopulation system. The evolutionary ecology of these bacterial systems in these synthetic ecosystems allows for using biophysics to address questions in evolutionary biology.

1.5.2.7. Cell behavior

The ability to create precise and carefully controlled chemoattractant gradients makes microfluidics the ideal tool to study motility, chemotaxis and the ability to evolve / develop resistance to antibiotics in small populations of microorganisms and in a short period of time. These microorganisms including bacteria and the broad range of organisms that form the marine microbial loop responsible for regulating much of the oceans' biogeochemistry, Microfluidics has also greatly aided the study of durotaxis by facilitating the creation of durotactic (stiffness) gradients.
1.5.2.8. Cellular biophysics

By rectifying the motion of individual swimming bacteria, microfluidic structures can be used to extract mechanical motion from a population of motile bacterial cells. This way, bacteria-powered rotors can be built.

1.5.2.9. Optics

The merger of microfluidics and optics is typically known as optofluidics. Examples of optofluidic devices are tunable microlens arrays and optofluidic microscopes. Microfluidic flow enables fast sample throughput, automated imaging of large sample populations, as well as 3D capabilities or super-resolution.

1.5.2.10. Acoustic droplet ejection (ADE)

Acoustic droplet ejection uses a pulse of ultrasound to move low volumes of fluids (typically nanoliters or picoliters) without any physical contact. This technology focuses acoustic energy into a fluid sample in order to eject droplets as small as a millionth of a millionth of a liter (picoliter = 10^{-12} litre). ADE technology is a very gentle process, and it can be used to transfer proteins, high molecular weight DNA and live cells without damage or loss of viability. This feature makes the technology suitable for a wide variety of applications including proteomics and cell-based assays.

1.5.2.11. Fuel cells

Microfluidic fuel cells can use laminar flow to separate the fuel and its oxidant to control the interaction of the two fluids without a physical barrier as would be required in conventional fuel cells.

1.5.2.12. Future directions

- On-chip characterization:
- Microfluidics in the classroom: On-chip acid-base titrations
CHAPTER 2: Bio-MEMS AS MINIATURIZED BIOSENSORS

2.1. Micromechanical sensors

Biosensors are devices that consist of a biological recognition system, called the bioreceptor, and a transducer. The interaction of the analyte with the bioreceptor causes an effect that the transducer can convert into a measurement, such as an electrical signal. The most common bioreceptors used in biosensing are based on antibody–antigen interactions, nucleic acid interactions, enzymatic interactions, cellular interactions, and interactions using biomimetic materials. Common transducer techniques include mechanical detection, electrical detection, and optical detection. Mechanical detection in bio-MEMS is achieved through micro- and nano-scale cantilevers for stress sensing and mass sensing, or micro- and nano-scale plates or membranes. In stress sensing, the biochemical reaction is performed selectively on one side of the cantilever to cause a change in surface free energy. This results in bending of the cantilever that is measurable either optically (laser reflection into a quadposition detector) or electrically (piezo-resistor at the fixed edge of the cantilever) due to a change in surface stress; In mass sensing, the cantilever vibrates at its resonant frequency as measured electrically or optically. When a biochemical reaction takes place and is captured on the cantilever, the mass of the cantilever changes, as does the resonant frequency. Mass sensing is not as effective in fluids because the minimum detectable mass is much higher in damped mediums, something that is overcome with plates or membranes. The advantage of using cantilever sensors is that there is no need for an optically detectable label on the analyte or bioreceptors.

2.2. Electrical and electrochemical sensors

Electrical and electrochemical detection are easily adapted for portability and miniaturization, especially in comparison to optical detection. In amperometric biosensors, an enzyme-catalyzed redox reaction causes a redox electron current that is measured by a working electrode. Amperometric biosensors have been used in bio-MEMS for detection of glucose, galactose, lactose, urea, and cholesterol, as well as for applications in gas detection and DNA hybridization. In potentiometric biosensors, measurements of electric potential at one electrode are made in reference to another electrode. Examples of potentiometric biosensors include ion-sensitive field
effect transistors (ISFET), Chemical field-effect transistors (chem-FET), and light-addressable potentiometric sensors (LAPS). In conductometric biosensors, changes in electrical impedance between two electrodes are measured as a result of a biomolecular reaction. Conductive measurements are simple and easy to use because there is no need for a specific reference electrode, and have been used to detect biochemicals, toxins, nucleic acids, and bacterial cells.

2.3. Optical sensors

A challenge in optical detection is the need for integrating detectors and photodiodes in a miniaturized portable format on the bio-MEMS. Optical detection includes fluorescence-based techniques, chemiluminescence-based techniques, and surface plasmon resonance (SPR). Fluorescence-based optical techniques use markers that emit light at specific wavelengths and the presence or enhancement/reduction (e.g. fluorescence resonance energy transfer) in optical signal indicates a reaction has occurred. Fluorescence-based detection has been used in microarrays and PCR on chip devices. Chemiluminescence is light generation by energy release from a chemical reaction. Bioluminescence and electrochemiluminescence are subtypes of chemiluminescence. Surface plasmon resonance sensors can be thin-film refractometers or gratings that measure the resonance behaviour of surface plasmon on metal or dielectric surfaces. The resonance changes when biomolecules are captured or adsorbed on the sensor surface and depends on the concentration of the analyte as well as its properties. Surface plasmon resonance has been used in food quality and safety analysis, medical diagnostics, and environmental monitoring.

A biosensor is an analytical device, used for the detection of an analyte that combines a biological component with a physicochemical detector. The *sensitive biological element* (e.g. tissue, microorganisms, organelles, cell receptors, enzymes, antibodies, nucleic acids, etc.) is a biologically derived material or biomimetic component that interacts (binds or recognizes) with the analyte under study. The biologically sensitive elements can also be created by biological engineering. The *transducer* or the *detector element* (works in a physicochemical way; optical, piezoelectric, electrochemical, etc.) transforms the signal resulting from the interaction of the analyte with the biological element into another signal (i.e., transduces) that can be more easily measured and quantified. The biosensor reader device with the associated electronics or signal
processors that are primarily responsible for the display of the results in a user-friendly way, this sometimes accounts for the most expensive part of the sensor device, however it is possible to generate a user friendly display that includes transducer and sensitive element (holographic sensor). The readers are usually custom-designed and manufactured to suit the different working principles of biosensors.

2.4. Biosensor system

A biosensor typically consists of a bio-recognition site, biotransducer component, and electronic system which include a signal amplifier, processor, and display. Transducers and electronics can be combined, e.g., in CMOS-based microsensor systems. The recognition component, often called a bioreceptor, uses biomolecules from organisms or receptors modeled after biological systems to interact with the analyte of interest. This interaction is measured by the biotransducer which outputs a measurable signal proportional to the presence of the target analyte in the sample. The general aim of the design of a biosensor is to enable quick, convenient testing at the point of concern or care where the sample was procured.

2.5. Bioreceptors

In a biosensor, the bioreceptor is designed to interact with the specific analyte of interest to produce an effect measurable by the transducer. High selectivity for the analyte among a matrix of other chemical or biological components is a key requirement of the bioreceptor. While the type of biomolecule used can vary widely, biosensors can be classified according to common type’s bioreceptor interactions involving: antibody/antigen, enzymes/ligands, nucleic acids/DNA, cellular structures/cells, or biomimetic materials.

2.5.1. Antibody/antigen interactions

An immunosensor utilizes the very specific binding affinity of antibodies for a specific compound or antigen. The specific nature of the antibody-antigen interaction is analogous to a lock and key fit in that the antigen will only bind to the antibody if it has the correct conformation. Binding events result in a physicochemical change that in combination with a tracer, such as a fluorescent molecules, enzymes, or radioisotopes, can generate a signal. There
are limitations with using antibodies in sensors: 1. The antibody binding capacity is strongly dependent on assay conditions (e.g. pH and temperature) and 2. The antibody-antigen interaction is generally irreversible. However, it has been shown that binding can be disrupted by chaotropic reagents, organic solvents, or even ultrasonic radiation.

2.5.2. Artificial binding proteins

The use of antibodies as the bio-recognition component of biosensors has several drawbacks. They have high molecular weights and limited stability, contain essential disulfide bonds and are expensive to produce. In one approach to overcome these limitations, recombinant binding fragments (Fab, Fv or scFv) or domains (VH, VHH) of antibodies have been engineered. In another approach, small protein scaffolds with favorable biophysical properties have been engineered to generate artificial families of Antigen Binding Proteins (AgBP), capable of specific binding to different target proteins while retaining the favorable properties of the parent molecule. The elements of the family that specifically bind to a given target antigen, are often selected in vitro by display techniques: phage display, ribosome display, yeast display or mRNA display. The artificial binding proteins are much smaller than antibodies (usually less than 100 amino-acid residues), have a strong stability, lack disulfide bonds and can be expressed in high yield in reducing cellular environments like the bacterial cytoplasm, contrary to antibodies and their derivatives. They are thus especially suitable to create biosensors.

2.5.3. Enzymatic interactions

The specific binding capabilities and catalytic activity of enzymes make them popular bioreceptors. Analyte recognition is enabled through several possible mechanisms: 1) the enzyme converting the analyte into a product that is sensor-detectable, 2) detecting enzyme inhibition or activation by the analyte, or 3) monitoring modification of enzyme properties resulting from interaction with the analyte. The main reasons for the common use of enzymes in biosensors are: 1) ability to catalyze a large number of reactions; 2) potential to detect a group of analytes (substrates, products, inhibitors, and modulators of the catalytic activity); and 3) suitability with several different transduction methods for detecting the analyte. Notably, since enzymes are not consumed in reactions, the biosensor can easily be used continuously. The
catalytic activity of enzymes also allows lower limits of detection compared to common binding techniques. However, the sensor's lifetime is limited by the stability of the enzyme.

2.5.4. Affinity binding receptors

Antibodies have a high binding constant in excess of $10^8$ L/mol, which stands for a nearly irreversible association once the antigen-antibody couple has formed. For certain analyte molecules like glucose affinity binding proteins exist that bind their ligand with a high specificity like an antibody, but with a much smaller binding constant on the order of $10^2$ to $10^4$ L/mol. The association between analyte and receptor then is of reversible nature and next to the couple between both also their free molecules occur in a measurable concentration. In case of glucose, for instance, concanavalin A may function as affinity receptor exhibiting a binding constant of $4 \times 10^2$ L/mol. The use of affinity binding receptors for purposes of biosensing has been proposed by Schultz and Sims in 1979 and was subsequently configured into a fluorescent assay for measuring glucose in the relevant physiological range between 4.4 and 6.1 mmol/L. The sensor principle has the advantage that it does not consume the analyte in a chemical reaction as is occurs in enzymatic assays.

2.5.5. Nucleic acid interactions

Biosensors that employ nucleic acid interactions can be referred to as genosensors. The recognition process is based on the principle of complementary base pairing, adenine:thymine and cytosine: guanine in DNA. If the target nucleic acid sequence is known, complementary sequences can be synthesized, labeled, and then immobilized on the sensor. The hybridization probes can then base pair with the target sequences, generating an optical signal. The favored transduction principle employed in this type of sensor has been optical detection.

2.5.6. Epigenetics

It has been proposed that properly optimized integrated optical resonators can be exploited for detecting epigenetic modifications (e.g. DNA methylation, histone post-translational modifications) in body fluids from patients affected by cancer or other diseases. Photonic biosensors with ultra-sensitivity are nowadays being developed at a research level to easily
detect cancerous cells within the patient's urine. Different research projects aim to develop new portable devices that use cheap, environmentally friendly, disposable cartridges that require only simple handling with no need of further processing, washing, or manipulation by expert technicians.

2.5.7. Organelles

Organelles form separate compartments inside cells and usually perform function independently. Different kinds of organelles have various metabolic pathways and contain enzymes to fulfill its function. Commonly used organelles include lysosome, chloroplast and mitochondria. The spatial-temporal distribution pattern of calcium is closely related to ubiquitous signaling pathway. Mitochondria actively participate in the metabolism of calcium ions to control the function and also modulate the calcium related signaling pathways. Experiments have proved that mitochondria have the ability to respond to high calcium concentration generated in the proximity by opening the calcium channel. In this way, mitochondria can be used to detect the calcium concentration in medium and the detection is very sensitive due to high spatial resolution. Another application of mitochondria is used for detection of water pollution. Detergent compounds' toxicity will damage the cell and subcellular structure including mitochondria. The detergents will cause a swelling effect which could be measured by an absorbance change. Experiment data shows the change rate is proportional to the detergent concentration, providing a high standard for detection accuracy.

2.5.8. Cells

Cells are often used in bioreceptors because they are sensitive to surrounding environment and they can respond to all kinds of stimulants. Cells tend to attach to the surface so they can be easily immobilized. Compared to organelles they remain active for longer period and the reproducibility makes them reusable. They are commonly used to detect global parameter like stress condition, toxicity and organic derivatives. They can also be used to monitor the treatment effect of drugs. One application is to use cells to determine herbicides which are main aquatic contaminant. Microalgae are entrapped on a quartz microfiber and the chlorophyll fluorescence modified by herbicides is collected at the tip of an optical fiber bundle and transmitted to a
fluorimeter. The algae are continuously cultured to get optimized measurement. Results show that detection limits of certain herbicide can reach sub-ppb concentration level. Some cells can also be used to monitor the microbial corrosion. Pseudomonas sp. is isolated form corroded material surface and immobilized on acetylcellulose membrane. The respiration activity is determined by measuring oxygen consumption. There is linear relationship between the current generated and the concentration of sulfuric acid. The response time is related to the loading of cells and surrounding environments and can be controlled to no more than 5min.

2.5.9. Tissue

Tissues are used for biosensor for the abundance of enzymes existed. Advantages of tissues as biosensors include the following: 1) easier to immobilize compared to cells and organelles 2) the higher activity and stability from maintain enzymes in natural environment 3) the availability and low price 4) the avoidance of tedious work of extraction, centrifuge and purification of enzymes 5) necessary cofactors for enzyme to function exists 6) the diversity providing a wide range of choice concerning different objectives. There also exist some disadvantages of tissues like the lack of specificity due to the interference of other enzymes and longer response time due to transport barrier.

2.6. Surface attachment of the biological elements

An important part in a biosensor is to attach the biological elements (small molecules/protein/cells) to the surface of the sensor (be it metal, polymer or glass). The simplest way is to functionalize the surface in order to coat it with the biological elements. This can be done by polylysine, aminosilane, epoxysilane or nitrocellulose in the case of silicon chips/silica glass. Subsequently, the bound biological agent may be for example fixed by Layer by layer deposition of alternatively charged polymer coatings. Alternatively three-dimensional lattices (hydrogel/xerogel) can be used to chemically or physically entrap these (where by chemically entraped it is meant that the biological element is kept in place by a strong bond, while physically they are kept in place being unable to pass through the pores of the gel matrix). The most commonly used hydrogel is sol-gel, a glassy silica generated by polymerization of silicate monomers (added as tetra alkyl orthosilicates, such as TMOS or TEOS) in the presence of the
biological elements (along with other stabilizing polymers, such as PEG) in the case of physical entrapment. Another group of hydrogels, which set under conditions suitable for cells or protein, are acrylate hydrogel, which polymerize upon radical initiation; One type of radical initiator is a peroxide radical, typically generated by combining a persulfate with TEMED (Polyacrylamide gel are also commonly used for protein electrophoresis), alternatively light can be used in combination with a photoinitiator, such as DMPA (2,2-dimethoxy-2-phenylacetophenone). Smart materials that mimic the biological components of a sensor can also be classified as biosensors using only the active or catalytic site or analogous configurations of a biomolecule.

2.7. Biotransducer

Biosensors can be classified by their biotransducer type. The most common types of biotransducers used in biosensors are 1) electrochemical biosensors, 2) optical biosensors, 3) electronic biosensors, 4) piezoelectric biosensors, 5) gravimetric biosensors, and 6) pyroelectric.

2.7.1. Electrochemical

Electrochemical biosensors are normally based on enzymatic catalysis of a reaction that produces or consumes electrons (such enzymes are rightly called redox enzymes). The sensor substrate usually contains three electrodes; a reference electrode, a working electrode and a counter electrode. The target analyte is involved in the reaction that takes place on the active electrode surface, and the reaction may cause either electron transfer across the double layer (producing a current) or can contribute to the double layer potential (producing a voltage). We can either measure the current (rate of flow of electrons is now proportional to the analyte concentration) at a fixed potential or the potential can be measured at zero current (this gives a logarithmic response). Note that potential of the working or active electrode is space charge sensitive and this is often used. Further, the label-free and direct electrical detection of small peptides and proteins is possible by their intrinsic charges using biofunctionalized ion-sensitive field-effect transistors.

Another example, the potentiometric biosensor, (potential produced at zero current) gives a logarithmic response with a high dynamic range. Such biosensors are often made by screen printing the electrode patterns on a plastic substrate, coated with a conducting polymer and then some protein (enzyme or antibody) is attached. They have only two electrodes and are extremely
sensitive and robust. They enable the detection of analytes at levels previously only achievable by HPLC and LC/MS and without rigorous sample preparation. All biosensors usually involve minimal sample preparation as the biological sensing component is highly selective for the analyte concerned. The signal is produced by electrochemical and physical changes in the conducting polymer layer due to changes occurring at the surface of the sensor. Such changes can be attributed to ionic strength, pH, hydration and redox reactions, and the latter due to the enzyme label turning over a substrate. Field effect transistors, in which the gate region has been modified with an enzyme or antibody, can also detect very low concentrations of various analytes as the binding of the analyte to the gate region of the FET cause a change in the drain-source current.

2.7.2. Ion channel switch

ICS – channel open

ICS – channel closed

The use of ion channels has been shown to offer highly sensitive detection of target biological molecules. By embedding the ion channels in supported or tethered bilayer membranes (t-BLM) attached to a gold electrode, an electrical circuit is created. Capture molecules such as antibodies can be bound to the ion channel so that the binding of the target molecule controls the ion flow through the channel. This results in a measurable change in the electrical conduction which is proportional to the concentration of the target. An ion channel switch (ICS) biosensor can be created using gramicidin, a dimeric peptide channel, in a tethered bilayer membrane. One peptide
of gramicidin, with attached antibody, is mobile and one is fixed. Breaking the dimer stops the ionic current through the membrane. The magnitude of the change in electrical signal is greatly increased by separating the membrane from the metal surface using a hydrophilic spacer. Quantitative detection of an extensive class of target species, including proteins, bacteria, drug and toxins has been demonstrated using different membrane and capture configurations.

2.7.3. Reagentless fluorescent biosensor

A reagentless biosensor can monitor a target analyte in a complex biological mixture without additional reagent. Therefore, it can function continuously if immobilized on a solid support. A fluorescent biosensor reacts to the interaction with its target analyte by a change of its fluorescence properties. A Reagentless Fluorescent biosensor (RF biosensor) can be obtained by integrating a biological receptor, which is directed against the target analyte, and a solvatochromic fluorophore, whose emission properties are sensitive to the nature of its local environment, in a single macromolecule. The fluorophore transduces the recognition event into a measurable optical signal. The use of extrinsic fluorophores, whose emission properties differ widely from those of the intrinsic fluorophores of proteins, tryptophan and tyrosine, enables one to immediately detect and quantify the analyte in complex biological mixtures. The integration of the fluorophore must be done in a site where it is sensitive to the binding of the analyte without perturbing the affinity of the receptor. Antibodies and artificial families of Antigen Binding Proteins (AgBP) are well suited to provide the recognition module of RF biosensors since they can be directed against any antigen (see the paragraph on bioreceptors). A general approach to integrate a solvatochromic fluorophore in an AgBP when the atomic structure of the complex with its antigen is known, and thus transform it into a RF biosensor, has been described. A residue of the AgBP is identified in the neighborhood of the antigen in their complex. This residue is changed into a cysteine by site-directed mutagenesis. The fluorophore is chemically coupled to the mutant cysteine. When the design is successful, the coupled fluorophore does not prevent the binding of the antigen, this binding shields the fluorophore from the solvent, and it can be detected by a change of fluorescence. This strategy is also valid for antibody fragments.

However, in the absence of specific structural data, other strategies must be applied. Antibodies and artificial families of AgBPs are constituted by a set of hypervariable (or randomized) residue
positions, located in a unique sub-region of the protein, and supported by a constant polypeptide scaffold. The residues that form the binding site for a given antigen are selected among the hypervariable residues. It is possible to transform any AgBP of these families into a RF biosensor, specific of the target antigen, simply by coupling a solvatochromic fluorophore to one of the hypervariable residues that have little or no importance for the interaction with the antigen, after changing this residue into cysteine by mutagenesis. More specifically, the strategy consists in individually changing the residues of the hypervariable positions into cysteine at the genetic level, in chemically coupling a solvatochromic fluorophore with the mutant cysteine, and then in keeping the resulting conjugates that have the highest sensitivity (a parameter that involves both affinity and variation of fluorescence signal). This approach is also valid for families of antibody fragments. A posteriori studies have shown that the best reagentless fluorescent biosensors are obtained when the fluorophore does not make non-covalent interactions with the surface of the bioreceptor, which would increase the background signal, and when it interacts with a binding pocket at the surface of the target antigen. The RF biosensors that are obtained by the above methods can function and detect target analytes inside living cells.

2.7.4. Others

Piezoelectric sensors utilize crystals which undergo an elastic deformation when an electrical potential is applied to them. An alternating potential (A.C.) produces a standing wave in the crystal at a characteristic frequency, This frequency is highly dependent on the elastic properties of the crystal, such that if a crystal is coated with a biological recognition element the binding of a (large) target analyte to a receptor will produce a change in the resonance frequency, which gives a binding signal. In a mode that uses surface acoustic waves (SAW), the sensitivity is greatly increased. This is a specialized application of the Quartz crystal microbalance as a biosensor. Thermometric and magnetic based biosensors are rare.

2.8. Placement of biosensors

The appropriate placement of biosensors depends on their field of application, which may roughly be divided into biotechnology, agriculture, food technology and biomedicine. In biotechnology, analysis of the chemical composition of cultivation broth can be conducted in-
line, on-line, at-line and off-line. As outlined by the US Food and Drug Administration (FDA) the sample is not removed from the process stream for in-line sensors, while it is diverted from the manufacturing process for on-line measurements. For at-line sensors the sample may be removed and analyzed in close proximity to the process stream. An example of the latter is the monitoring of lactose in a dairy processing plant. Off-line biosensors compare to bioanalytical techniques that are not operating in the field, but in the laboratory. These techniques are mainly used in agriculture, food technology and biomedicine.

In medical applications biosensors are generally categorized as in vitro and in vivo systems. An in vitro biosensor measurement takes place in a test tube, a culture dish, a microtiter plate or elsewhere outside a living organism. The sensor uses a bioreceptor and transducer as outlined above. An example of an in vitro biosensor is an enzyme-conductimetric biosensor for blood glucose monitoring. There is a challenge to create a biosensor that operates by the principle of Point-of-care testing, i.e. at the location where the test is needed. The elimination of lab testing can save time and money. An application of a POCT biosensor can be for the testing of HI virus in areas, where it is difficult for patients to be tested. A biosensor can be sent directly to the location and a quick and easy test can be used.

![Medical biosensor implant for glucose monitoring in subcutaneous tissue (59x45x8 mm)](image)

Electronic components like microcontroller, radio chip etc. are hermetically enclosed in a Ti casing, while ring-shaped antenna and top-most sensor probe are moulded into the transparent epoxy header.

An in vivo biosensor is an implantable device that operates inside the body. Of course, biosensor implants have to fulfill the strict regulations on sterilization in order to avoid an initial
inflammatory response after implantation. The second concern relates to the long-term biocompatibility, i.e. the unharmful interaction with the body environment during the intended period of use. Another issue that arises is failure. If there is failure, the device must be removed and replaced, causing additional surgery. An example for application of an in vivo biosensor would be the insulin monitoring within the body, which is not available yet. Most advanced biosensor implants have been developed for the continuous monitoring of glucose. The figure displays a device, for which a Ti casing and a battery as established for cardiovascular implants like pacemakers and defibrillators is used. Its size is determined by the battery as required for a lifetime of one year. Measured glucose data will be transmitted wirelessly out of the body within the MICS 402-405 MHz band as approved for medical implants. Nowadays biosensors are being integrated into mobile phone systems, making them user-friendly and accessible to a large amount of users.

2.9. Applications

There are many potential applications of biosensors of various types. The main requirements for a biosensor approach to be valuable in terms of research and commercial applications are the identification of a target molecule, availability of a suitable biological recognition element, and the potential for disposable portable detection systems to be preferred to sensitive laboratory-based techniques in some situations. Some examples are glucose monitoring in diabetes patients, other medical health related targets, environmental applications e.g. the detection of pesticides and river water contaminants such as heavy metal ions, remote sensing of airborne bacteria e.g. in counter-bioterrorist activities, remote sensing of water quality in coastal waters by describing online different aspects of clam ethology (biological rhythms, growth rates, spawning or death records) in groups of abandoned bivalves around the world, detection of pathogens, determining levels of toxic substances before and after bioremediation, detection and determining of organophosphate, routine analytical measurement of folic acid, biotin, vitamin B12 and pantothenic acid as an alternative to microbiological assay, determination of drug residues in food, such as antibiotics and growth promoters, particularly meat and honey, drug discovery and evaluation of biological activity of new compounds, protein engineering in biosensors, and detection of toxic metabolites such as mycotoxins.
A common example of a commercial biosensor is the blood glucose biosensor, which uses the enzyme glucose oxidase to break blood glucose down. In doing so it first oxidizes glucose and uses two electrons to reduce the FAD (a component of the enzyme) to FADH2. This in turn is oxidized by the electrode in a number of steps. The resulting current is a measure of the concentration of glucose. In this case, the electrode is the transducer and the enzyme is the biologically active component. A canary in a cage, as used by miners to warn of gas, could be considered a biosensor. Many of today's biosensor applications are similar, in that they use organisms which respond to toxic substances at much lower concentrations than humans can detect to warn of their presence. Such devices can be used in environmental monitoring, trace gas detection and in water treatment facilities. Many optical biosensors are based on the phenomenon of surface plasmon resonance (SPR) techniques. This utilizes a property of and other materials; specifically that a thin layer of gold on a high refractive index glass surface can absorb laser light, producing electron waves (surface plasmons) on the gold surface. This occurs only at a specific angle and wavelength of incident light and is highly dependent on the surface of the gold, such that binding of a target analyte to a receptor on the gold surface produces a measurable signal.

Surface plasmon resonance sensors operate using a sensor chip consisting of a plastic cassette supporting a glass plate, one side of which is coated with a microscopic layer of gold. This side contacts the optical detection apparatus of the instrument. The opposite side is then contacted with a microfluidic flow system. The contact with the flow system creates channels across which reagents can be passed in solution. This side of the glass sensor chip can be modified in a number of ways, to allow easy attachment of molecules of interest. Normally it is coated in carboxymethyl dextran or similar compound. The refractive index at the flow side of the chip surface has a direct influence on the behavior of the light reflected off the gold side. Binding to the flow side of the chip has an effect on the refractive index and in this way biological interactions can be measured to a high degree of sensitivity with some sort of energy. The refractive index of the medium near the surface changes when biomolecules attach to the surface, and the SPR angle varies as a function of this change. Light of a fixed wavelength is reflected off the gold side of the chip at the angle of total internal reflection, and detected inside the instrument. The angle of incident light is varied in order to match the evanescent wave
propagation rate with the propagation rate of the surface plasmon plaritons. This induces the evanescent wave to penetrate through the glass plate and some distance into the liquid flowing over the surface.

Other optical biosensors are mainly based on changes in absorbance or fluorescence of an appropriate indicator compound and do not need a total internal reflection geometry. For example, a fully operational prototype device detecting casein in milk has been fabricated. The device is based on detecting changes in absorption of a gold layer. A widely used research tool, the micro-array, can also be considered a biosensor. Biological biosensors often incorporate a genetically modified form of a native protein or enzyme. The protein is configured to detect a specific analyte and the ensuing signal is read by a detection instrument such as a fluorometer or luminometer. An example of a recently developed biosensor is one for detecting cytosolic concentration of the analyte cAMP (cyclic adenosine monophosphate), a second messenger involved in cellular signaling triggered by ligands interacting with receptors on the cell membrane. Similar systems have been created to study cellular responses to native ligands or xenobiotics (toxins or small molecule inhibitors). Such "assays" are commonly used in drug discovery development by pharmaceutical and biotechnology companies. Most cAMP assays in current use require lysis of the cells prior to measurement of cAMP. A live-cell biosensor for cAMP can be used in non-lysed cells with the additional advantage of multiple reads to study the kinetics of receptor response. Nanobiosensors use an immobilized bioreceptor probe that is selective for target analyte molecules. Nanomaterials are exquisitely sensitive chemical and biological sensors. Nanoscale materials demonstrate unique properties. Their large surface area to volume ratio can achieve rapid and low cost reactions, using a variety of designs.

Other evanescent wave biosensors have been commercialised using waveguides where the propagation constant through the waveguide is changed by the absorption of molecules to the waveguide surface. One such example, dual polarisation interferometry uses a buried waveguide as a reference against which the change in propagation constant is measured. Other configurations such as the Mach–Zehnder have reference arms lithographically defined on a substrate. Higher levels of integration can be achieved using resonator geometries where the resonant frequency of a ring resonator changes when molecules are absorbed. Recently, arrays of many different detector molecules have been applied in so called electronic nose devices, where
the pattern of response from the detectors is used to fingerprint a substance. In the Wasp Hound odor-detector, the mechanical element is a video camera and the biological element is five parasitic wasps that have been conditioned to swarm in response to the presence of a specific chemical. Current commercial electronic noses, however, do not use biological elements.

2.9.1. Glucose monitoring

Commercially available glucose monitors rely on amperometric sensing of glucose by means of glucose oxidase, which oxidises glucose producing hydrogen peroxide which is detected by the electrode. To overcome the limitation of amperometric sensors, a flurry of research is present into novel sensing methods, such as fluorescent glucose biosensors. Blood glucose monitoring is a way of testing the concentration of glucose in the blood (glycemia). Particularly important in diabetes management, a blood glucose test is typically performed by piercing the skin (typically, on the finger) to draw blood, then applying the blood to a chemically active disposable 'test-strip'. Different manufacturers use different technology, but most systems measure an electrical characteristic, and use this to determine the glucose level in the blood. The test is usually referred to as capillary blood glucose. Healthcare professionals advise patients with diabetes mellitus on the appropriate monitoring regimen for their condition. Most people with type 2 diabetes test at least once per day. The Mayo Clinic generally recommends that diabetics who use insulin (all type 1 diabetics and many type 2 diabetics) test their blood sugar more often (4-8 times per day for type 1 diabetics, 2 or more times per day for type 2 diabetics), both to assess the effectiveness of their prior insulin dose and to help determine their next insulin dose.

2.9.1.1. Purpose

Blood glucose monitoring reveals individual patterns of blood glucose changes, and helps in the planning of meals, activities, and at what time of day to take medications. Also, testing allows for quick response to high blood sugar (hyperglycemia) or low blood sugar (hypoglycemia). This might include diet adjustments, exercise, and insulin (as instructed by the health care provider).
2.9.1.2. Blood glucose meters

Four generations of blood glucose meter, c. 1991–2005. Sample sizes vary from 30 to 0.3 μl. Test times vary from 5 seconds to 2 minutes (modern meters are typically below 15 seconds). A blood glucose meter is an electronic device for measuring the blood glucose level. A relatively small drop of blood is placed on a disposable test strip which interfaces with a digital meter. Within several seconds, the level of blood glucose will be shown on the digital display.

Needing only a small drop of blood for the meter means that the time and effort required for testing is reduced and the compliance of diabetic people to their testing regimens is improved. Although the cost of using blood glucose meters seems high, it is believed to be a cost benefit relative to the avoided medical costs of the complications of diabetes.

Recent advances include:

- **Alternate site testing**, the use of blood drops from places other than the finger, usually the palm or forearm. This alternate site testing uses the same test strips and meter, is practically pain free, and gives the real estate on the finger tips a needed break if they become sore. The disadvantage of this technique is that there is usually less blood flow to alternate sites, which prevents the reading from being accurate when the blood sugar level is changing.

- **No coding' systems.** Older systems required 'coding' of the strips to the meter. This carried a risk of 'miscoding', which can lead to inaccurate results. Two approaches have resulted in systems that no longer require coding. Some systems are 'auto coded', where technology is used to code each strip to the meter. And some are manufactured to a 'single code', thereby avoiding the risk of miscoding.
**Multi-test** systems. Some systems use a cartridge or a disc containing multiple test strips. This has the advantage that the user doesn't have to load individual strips each time, which is convenient and can enable quicker testing.

**Downloadable** meters. Most new systems come with software that allows the user to download meter results to a computer. This information can then be used, together with health care professional guidance, to enhance and improve diabetes management. The meters usually require a connection cable, unless they are designed to work wirelessly with an insulin pump, or are designed to plug directly into the computer.

### 2.9.1.3. Continuous glucose monitoring

A continuous glucose monitor (CGM) determines glucose levels on a continuous basis (every few minutes). A typical system consists of:

- a disposable glucose sensor placed just under the skin, which is worn for a few days until replacement
- a link from the sensor to a non-implanted transmitter which communicates to a radio receiver
- An electronic receiver worn like a pager (or insulin pump) that displays glucose levels with nearly continuous updates, as well as monitors rising and falling trends.

Continuous glucose monitors measure the concentration of glucose in a sample of interstitial fluid. Shortcomings of CGM systems due to this fact are:

- continuous systems must be calibrated with a traditional blood glucose measurement (using current technology) and therefore require both the CGM system and occasional "fingerstick"
- glucose levels in interstitial fluid lag behind blood glucose values

Patients therefore require traditional fingerstick measurements for calibration (typically twice per day) and are often advised to use fingerstick measurements to confirm hypo- or hyperglycemia before taking corrective action.

The lag time discussed above has been reported to be about 5 minutes. Anecdotally, some users of the various systems report lag times of up to 10–15 minutes. This lag time is insignificant when blood sugar levels are relatively consistent. However, blood sugar levels, when changing
rapidly, may read in the normal range on a CGM system while in reality the patient is already experiencing symptoms of an out-of-range blood glucose value and may require treatment. Patients using CGM are therefore advised to consider both the absolute value of the blood glucose level given by the system as well as any trend in the blood glucose levels. For example, a patient using CGM with a blood glucose of 100 mg/dl on their CGM system might take no action if their blood glucose has been consistent for several readings, while a patient with the same blood glucose level but whose blood glucose has been dropping steeply in a short period of time might be advised to perform a fingerstick test to check for hypoglycemia.

Continuous monitoring allows examination of how the blood glucose level reacts to insulin, exercise, food, and other factors. The additional data can be useful for setting correct insulin dosing ratios for food intake and correction of hyperglycemia. Monitoring during periods when blood glucose levels are not typically checked (e.g. overnight) can help to identify problems in insulin dosing (such as basal levels for insulin pump users or long-acting insulin levels for patients taking injections). Monitors may also be equipped with alarms to alert patients of hyperglycemia or hypoglycemia so that a patient can take corrective action(s) (after fingerstick testing, if necessary) even in cases where they do not feel symptoms of either condition. While the technology has its limitations, studies have demonstrated that patients with continuous sensors experience less hyperglycemia and also reduce their glycosylated hemoglobin levels.

Currently, continuous blood glucose monitoring is not automatically covered by health insurance in the United States in the same way that most other diabetic supplies are covered (e.g. standard glucose testing supplies, insulin, and even insulin pumps). However, an increasing number of insurance companies do cover continuous glucose monitoring supplies (both the receiver and disposable sensors) on a case-by-case basis if the patient and doctor show a specific need. The lack of insurance coverage is exacerbated by the fact that disposable sensors must be frequently replaced. Some sensors have been U.S. Food and Drug Administration (FDA) approved for 7- and 3-day use, though some patients wear sensors for longer than the recommended period) and the receiving meters likewise have finite lifetimes (less than 2 years and as little as 6 months). This is one factor in the slow uptake in the use of sensors that have been marketed in the United States. The principles, history and recent developments of operation of electrochemical glucose biosensors are discussed in a chemical review by Joseph Wang. Some
projects such Nightscout (DIY) allow parents to have a permanent control of glucose levels on their children from devices such cell phones, tablets, laptops, pebble watch, etc.

2.9.1.4. Glucose sensing bio-Implants

Investigations on the use of test strips have shown that the required self-injury acts as a psychological barrier restraining the patients from sufficient glucose control. As a result, secondary diseases are caused by excessive glucose levels. A significant improvement of diabetes therapy might be achieved with an implantable sensor that would continuously monitor blood sugar levels within the body and transmit the measured data outside. The burden of regular blood testing would be taken from the patient, who would instead follow the course of their glucose levels on an intelligent device like a laptop or a smart phone. Glucose concentrations do not necessarily have to be measured in blood vessels, but may also be determined in the interstitial fluid, where the same levels prevail – with a time lag of a few minutes – due to its connection with the capillary system. However, the enzymatic glucose detection scheme used in single-use test strips is not directly suitable for implants. One main problem is caused by the varying supply of oxygen, by which glucose is converted to glucono lactone and \( \text{H}_2\text{O}_2 \) by glucose oxidase. Since the implantation of a sensor into the body is accompanied by growth of encapsulation tissue, the diffusion of oxygen to the reaction zone is continuously diminished. This decreasing oxygen availability causes the sensor reading to drift, requiring frequent re-calibration using finger-sticks and test strips.

One approach to achieving long-term glucose sensing is to measure and compensate for the changing local oxygen concentration. Other approaches replace the troublesome glucose oxidase reaction with a reversible sensing reaction, known as an affinity assay. This scheme was originally put forward by Schultz & Sims in 1978. A number of different affinity assays have been investigated, with fluorescent assays proving most common. MEMS technology has recently allowed for smaller and more convenient alternatives to fluorescent detection, via measurement of viscosity. Investigation of affinity-based sensors has shown that encapsulation by body tissue does not cause a drift of the sensor signal, but only a time lag of the signal compared to the direct measurement in blood.
2.9.1.5. Non-invasive technologies

Some new technologies to monitor blood glucose levels will not require access to blood to read the glucose level. Non-invasive technologies include near IR detection, ultrasound and dielectric spectroscopy. These may free the person with diabetes from finger sticks to supply the drop of blood for blood glucose analysis. Most of the non-invasive methods under development are continuous glucose monitoring methods and offer the advantage of providing additional information to the subject between the conventional finger stick, blood glucose measurements and over time periods where no finger stick measurements are available (i.e. while the subject is sleeping).

2.9.1.6. Effectiveness

For patients with diabetes mellitus type 2, the importance of monitoring and the optimal frequency of monitoring are not clear. A 2011 study found no evidence that blood glucose monitoring leads to better patient outcomes in actual practice. One randomized controlled trial found that self-monitoring of blood glucose did not improve glycosylated hemoglobin (HbA1c) among "reasonably well controlled non-insulin treated patients with type 2 diabetes". However a recent meta-analysis of 47 randomized controlled trials encompassing 7677 patients showed that self-care management intervention improves glycemic control in diabetics, with an estimated 0.36% (95% CI, 0.21-0.51) reduction in their glycosylated hemoglobin values. Furthermore, a recent study showed that patients described as being "Uncontrolled Diabetics" (defined in this study by HbA1C levels >8%) showed a statistically significant decrease in the HbA1C levels after a 90-day period of seven-point self-monitoring of blood glucose (SMBG) with a relative risk reduction (RRR) of 0.18% (95% CI, 0.86-2.64%, p<.001). Regardless of lab values or other numerical parameters, the purpose of the clinician is to improve quality of life and patient outcomes in diabetic patients.

A recent study included 12 randomized controlled trials and evaluated outcomes in 3259 patients. The authors concluded through a qualitative analysis that SMBG on quality of life showed no effect on patient satisfaction or the patients' health-related quality of life. Furthermore, the same study identified that patients with type 2 diabetes mellitus diagnosed greater than one year prior to initiation of SMBG, who were not on insulin, experienced a
statistically significant reduction in their HbA1C of 0.3% (95% CI, -0.4 - -0.1) at six months follow up, but a statistically insignificant reduction of 0.1% (95% CI, -0.3 – 0.04) at twelve months follow up. Conversely, newly diagnosed patients experienced a statistically significant reduction of 0.5% (95% CI, -0.9 – -0.1) at 12 months follow up. A recent study found that a treatment strategy of intensively lowering blood sugar levels (below 6%) in patients with additional cardiovascular disease risk factors poses more harm than benefit. For type 2 diabetics who are not on insulin, exercise and diet are the best tools. Blood glucose monitoring is, in that case, simply a tool to evaluate the success of diet and exercise. Insulin-dependent type 2 diabetics do not need to monitor their blood sugar as frequently as type 1 diabetics.

2.9.2. Interferometric reflectance imaging sensor

The interferometric reflectance imaging sensor (IRIS) is based on the principles of optical interference and consists of a silicon-silicon oxide substrate, standard optics, and low-powered coherent LEDs. When light is illuminated through a low magnification objective onto the layered silicon-silicon oxide substrate, an interferometric signature is produced. As biomass, which has a similar index of refraction as silicon oxide, accumulates on the substrate surface, a change in the interferometric signature occurs and the change can be correlated to a quantifiable mass. Daaboul et al. used IRIS to yield a label-free sensitivity of approximately 19 ng/mL. Ahn et al. improved the sensitivity of IRIS through a mass tagging technique.

Since initial publication, IRIS has been adapted to perform various functions. First, IRIS integrated a fluorescence imaging capability into the interferometric imaging instrument as a potential way to address fluorescence protein microarray variability. Briefly, the variation in fluorescence microarrays mainly derives from inconsistent protein immobilization on surfaces and may cause misdiagnoses in allergy microarrays. To correct from any variation in protein immobilization, data acquired in the fluorescence modality is then normalized by the data acquired in the label-free modality. IRIS has also been adapted to perform single nanoparticle counting by simply switching the low magnification objective used for label-free biomass quantification to a higher objective magnification. This modality enables size discrimination in complex human biological samples. Monroe et al. used IRIS to quantify protein levels spiked into human whole blood and serum and determined allergen sensitization in characterized human
blood samples using zero sample processing. Other practical uses of this device include virus and pathogen detection.

2.9.3. Food analysis

There are several applications of biosensors in food analysis. In the food industry, optics coated with antibodies is commonly used to detect pathogens and food toxins. Commonly, the light system in these biosensors is fluorescence, since this type of optical measurement can greatly amplify the signal. A range of immuno- and ligand-binding assays for the detection and measurement of small molecules such as water-soluble vitamins and chemical contaminants (drug residues) such as sulfonamides and Beta-agonists have been developed for use on SPR based sensor systems, often adapted from existing ELISA or other immunological assay. These are in widespread use across the food industry.

2.9.4. DNA biosensors

In the future, DNA will find use as a versatile material from which scientists can craft biosensors. DNA biosensors can theoretically be used for medical diagnostics, forensic science, agriculture, or even environmental clean-up efforts. No external monitoring is needed for DNA-based sensing devices. This is a significant advantage. DNA biosensors are complicated mini-machines consisting of sensing elements, micro lasers, and a signal generator. At the heart of DNA biosensor function is the fact that two strands of DNA stick to each other by virtue of chemical attractive forces. On such a sensor, only an exact fit that is, two strands that match up at every nucleotide position gives rise to a fluorescent signal (a glow) that is then transmitted to a signal generator.

2.9.5. Microbial biosensors

Using biological engineering researchers have created many microbial biosensors. An example is the arsenic biosensor. To detect arsenic they use the Ars operon. Using bacteria, researchers can detect pollutants in samples.
2.9.6. Ozone biosensors

Because ozone filters out harmful ultraviolet radiation, the discovery of holes in the ozone layer of the earth's atmosphere has raised concern about how much ultraviolet light reaches the earth's surface. Of particular concern are the questions of how deeply into sea water ultraviolet radiation penetrates and how it affects marine organisms, especially plankton (floating microorganisms) and viruses that attack plankton. Plankton forms the base of the marine food chains and is believed to affect our planet's temperature and weather by uptake of CO2 for photosynthesis. Deneb Karentz, a researcher at the Laboratory of Radio-biology and Environmental Health (University of California, San Francisco) has devised a simple method for measuring ultraviolet penetration and intensity. Working in the Antarctic Ocean, she submerged to various depths thin plastic bags containing special strains of *E. coli* that are almost totally unable to repair ultraviolet radiation damage to their DNA. Bacterial death rates in these bags were compared with rates in unexposed control bags of the same organism. The bacterial "biosensors" revealed constant significant ultraviolet damage at depths of 10 m and frequently at 20 and 30 m. Karentz plans additional studies of how ultraviolet may affect seasonal plankton blooms (growth spurts) in the oceans.

2.9.7. Metastatic cancer cell biosensors

Metastasis is the spread of cancer from one part of the body to another via either the circulatory system or lymphatic system. Unlike radiology imaging tests (mammograms), which send forms of energy (x-rays, magnetic fields, etc.) through the body to only take interior pictures, biosensors have the potential to directly test the malignant power of the tumor. The combination of a biological and detector element allows for a small sample requirement, a compact design, rapid signals, rapid detection, high selectivity and high sensitivity for the analyte being studied. Compared to the usual radiology imaging tests biosensors have the advantage of not only finding out how far the cancer has spread and checking if treatment is effective, but also are cheaper, more efficient (in time, cost and productivity) ways to assess metastaticity in early stages of cancer. Biological engineering researchers have created oncological biosensors for breast cancer. Breast cancer is the leading common cancer among women worldwide. An example would be a transferrin- quartz crystal microbalance (QCM). As a biosensor, quartz crystal microbalances
produce oscillations in the frequency of the crystal’s standing wave from an alternating potential to detect nano-gram mass changes. These biosensors are specifically designed to interact and have high selectivity for receptors on cell (cancerous and normal) surfaces. Ideally this provides a quantitative detection of cells with this receptor per surface area instead of a qualitative picture detection given by mammograms.

Seda Atay, a biotechnology researcher at Hacettepe University, experimentally observed this specificity and selectivity between a QCM and MDA-MB 231 breast cells, MCF 7 cells, and starved MDA-MB 231 cells in vitro. With other researchers she devised a method of washing these different metastatic leveled cells over the sensors to measure mass shifts due to different quantities of transferrin receptors. Particularly, the metastatic power of breast cancer cells can be determined by Quartz crystal microbalances with nanoparticles and transferrin that would potentially attach to transferrin receptors on cancer cell surfaces. There is very high selectivity for transferrin receptors because they are over-expressed in cancer cells. If cells have high expression of transferrin receptors, which shows their high metastatic power, they have higher affinity and bind more to the QCM that measures the increase in mass. Depending on the magnitude of the nano-gram mass change, the metastatic power can be determined.

2.9.7.1. Purpose of nanoparticles

When nanoparticles are attached to the QCM surface their simplicity, variability in shape, high surface area, physicochemical malleability, and optional attachment of metals enables for different properties, a change in responses, selectivities and specificities. This transducer’s characteristics in combination with nanoparticles with large surface area to volume ratios make it a perfect biosensor to particularly determine the metastatic power and malignancy of cancer cells. In Seda Atay’s study, determining the metastatic power of in vitro breast cancer exactly required 58 nm sized Poly(2-hydroxyethyl methacrylate) (PHEMA) nanoparticles with a surface area of 1899 m2g-1 to effectively adsorb the cells to the QCM surface.
CHAPTER 3: Bio-MEMS FOR DIAGNOSTICS

3.1. Genomic and proteomic microarrays

Affymetrix GeneChip is an example of a genomic microarray

The goals of genomic and proteomic microarrays are to make high-throughput genome analysis faster and cheaper, as well as identify activated genes and their sequences. There are many different types of biological entities used in microarrays, but in general the microarray consists of an ordered collection of microspots each containing a single defined molecular species that interacts with the analyte for simultaneous testing of thousands of parameters in a single experiment. Some applications of genomic and proteomic microarrays are neonatal screening, identifying disease risk, and predicting therapy efficacy for personalized medicine.

3.1.1. Oligonucleotide chips

Oligonucleotide chips are microarrays of oligonucleotides. They can be used for detection of mutations and expression monitoring, and gene discovery and mapping. The main methods for creating an oligonucleotide microarray are by gel pads (Motorola), microelectrodes (Nanogen), photolithography (Affymetrix), and inkjet technology (Agilent).

- Using gel pads, prefabricated oligonucleotides are attached to patches of activated polyacrylamide
• Using microelectrodes, negatively charged DNA and molecular probes can be concentrated on energized electrodes for interaction
• Using photolithography, a light exposure pattern is created on the substrate using a photomask or virtual photomask projected from a digital micromirror device. The light removes photolabile protecting groups from the selected exposure areas. Following de-protection, nucleotides with a photolabile protecting group are exposed to the entire surface and the chemical coupling process only occurs where light was exposed in the previous step. This process can be repeated to synthesize oligonucleotides of relatively short lengths on the surface, nucleotide by nucleotide.
• Using inkjet technology, nucleotides are printed onto a surface drop by drop to form oligonucleotides.

3.1.2. cDNA microarray

Differential comparison in cDNA microarray

cDNA microarrays are often used for large-scale screening and expression studies. In cDNA microarrays, mRNA from cells are collected and converted into cDNA by reverse transcription. Subsequently, cDNA molecules (each corresponding to one gene) are immobilized as ~100 µm diameter spots on a membrane, glass, or silicon chip by metallic pins. For detection,
fluorescently-labelled single strand cDNA from cells hybridize to the molecules on the microarray and a differential comparison between a treated sample (labelled red, for example) and an untreated sample (labelled in another color such as green) is used for analysis. Red dots mean that the corresponding gene was expressed at a higher level in the treated sample. Conversely, green dots mean that the corresponding gene was expressed at a higher level in the untreated sample. Yellow dots, as a result of the overlap between red and green dots, mean that the corresponding gene was expressed at relatively the same level in both samples, whereas dark spots indicate no or negligible expression in either sample.

### 3.1.3. Peptide and protein microarrays

The motivation for using peptide and protein microarrays is firstly because mRNA transcripts often correlate poorly with the actual amount of protein synthesized. Secondly, DNA microarrays cannot identify post-translational modification of proteins, which directly influences protein function. Thirdly, some bodily fluids such as urine lack mRNA. A protein microarray consists of a protein library immobilized on a substrate chip, usually glass, silicon, polystyrene, PVDF, or nitrocellulose. In general, there are three types of protein microarrays: functional, analytical or capture, and reverse-phase protein arrays.

- **Functional protein arrays** display folded and active proteins and are used for screening molecular interactions, studying protein pathways, identifying targets for post-translational modification, and analyzing enzymatic activities.
- **Analytical or capture protein arrays** display antigens and antibodies to profile protein or antibody expression in serum. These arrays can be used for biomarker discovery, monitoring of protein quantities, monitoring activity states in signalling pathways, and profiling antibody repertoires in diseases.
- **Reverse-phase protein arrays** test replicates of cell lysates and serum samples with different antibodies to study the changes in expression of specific proteins and protein modifications during disease progression, as well as biomarker discovery.

Protein microarrays have stringent production, storage, and experimental conditions due to the low stability and necessity of considering the native folding on the immobilized proteins.
Peptides, on the other hand, are more chemically resistant and can retain partial aspects of protein function. As such, peptide microarrays have been used to complement protein microarrays in proteomics research and diagnostics. Protein microarrays usually use *Escherichia coli* to produce proteins of interest; whereas peptide microarrays use the SPOT technique (stepwise synthesis of peptides on cellulose) or photolithography to make peptides.

### 3.2. PCR chips

Continuous flow-based PCR microfluidic system with thin film heaters, syringe pump, and continuous flow PCR channel; The application of this example bio-MEMS is for amplification of influenza A RNA in respiratory specimens.

The polymerase chain reaction (PCR) is a fundamental molecular biology technique that enables the selective amplification of DNA sequences, which is useful for expanded use of rare samples e.g.: stem cells, biopsies, circulating tumor cells. The reaction involves thermal cycling of the DNA sequence and DNA polymerase through three different temperatures. Heating up and cooling down in conventional PCR devices are time-consuming and typical PCR reactions can take hours to complete. Other drawbacks of conventional PCR is the high consumption of expensive reagents, preference for amplifying short fragments, and the production of short chimeric molecules. PCR chips serves to miniaturize PCR into microfluidic bio-MEMS and can achieve rapid heat transfer and fast mixing due to the larger surface-to-volume ratio and short diffusion distances respectively. The advantages of PCR chips include shorter thermal-cycling time, more uniform temperatures during the PCR process for enhanced yield, and portability for
point-of-care applications. Two challenges in microfluidic PCR chips are PCR inhibition and contamination due to the large surface-to-volume ratio increasing surface-reagent interactions. For example, silicon substrates have good thermal conductivity for rapid heating and cooling, but can poison the polymerase reaction. There are stationary (chamber-based), dynamic (continuous flow-based), and microdroplet (digital PCR) chip architectures.

- Chamber-based architecture is the result of shrinking down of conventional PCR reactors, which is difficult to scale up. A four-layer glass-PDMS device has been developed using this architecture integrating microvalves, microheaters, temperature sensors, 380-nL reaction chambers, and capillary electrophoresis channels for reverse transcription polymerase chain reaction (RT-PCR) that has attomolar detection sensitivity.
- Continuous flow-based architecture moves the sample through different temperature zones to achieve thermal cycling. This approach uses less energy and has high throughput, but has large reagent consumption and gas bubbles can form inside the flow channels.
- Digital PCR eliminates sample/reagent surface adsorption and contamination by carrying out PCR in microdroplets or microchambers. PCR in droplets also prevents recombination of homologous gene fragments so synthesis of short chimeric products is eliminated.

3.2.1. Polymerase chain reaction

A strip of eight PCR tubes, each containing a 100 μl reaction mixture
Polymerase chain reaction (PCR) is a technique used in molecular biology to amplify a single copy or a few copies of a segment of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence. It is an easy, cheap, and reliable way to repeatedly replicate a focused segment of DNA, a concept which is applicable to numerous fields in modern biology and related sciences. Developed in 1983 by Kary Mullis, PCR is now a common and often indispensable technique used in clinical and research laboratories for a broad variety of applications. These include DNA cloning for sequencing, gene cloning and manipulation, gene mutagenesis; construction of DNA-based phylogenies, or functional analysis of genes; diagnosis and monitoring of hereditary diseases; amplification of ancient DNA; analysis of genetic fingerprints for DNA profiling (for example, in forensic science and parentage testing); and detection of pathogens in nucleic acid tests for the diagnosis of infectious diseases. In 1993, Mullis was awarded the Nobel Prize in Chemistry along with Michael Smith for his work on PCR.

*Placing a strip of eight PCR tubes into a thermal cycler*

The vast majority of PCR methods rely on thermal cycling, which involves exposing the reactants to cycles of repeated heating and cooling, permitting different temperature-dependent reactions specifically, DNA melting and enzyme-driven DNA replication to quickly proceed many times in sequence. Primers (short DNA fragments) containing sequences complementary to the target region, along with a DNA polymerase, after which the method is named, enable selective and repeated amplification. As PCR progresses, the DNA generated is itself used as a template for replication, setting in motion a chain reaction in which the original DNA template is exponentially amplified. The simplicity of the basic principle underlying PCR means it can be extensively modified to perform a wide array of genetic manipulations. PCR is not generally considered to be a recombinant DNA method, as it does not involve cutting and pasting DNA,
only amplification of existing sequences. Almost all PCR applications employ a heat-stable DNA polymerase, such as Taq polymerase, an enzyme originally isolated from the thermophilic bacterium *Thermus aquaticus*. This DNA polymerase enzymatically assembles a new DNA strand from free nucleotides, the building blocks of DNA, by using single-stranded DNA as a template and DNA oligonucleotides (the primers mentioned above) to initiate DNA synthesis. In the first step, the two strands of the DNA double helix are physically separated at a high temperature in a process called DNA melting. In the second step, the temperature is lowered and the two DNA strands become templates for DNA polymerase to selectively amplify the target DNA. The selectivity of PCR results from the use of primers that are complementary to sequence around the DNA region targeted for amplification under specific thermal cycling conditions

3.2.2. Principles

A thermal cycler for PCR

*An older model three-temperature thermal cycler for PCR*
PCR amplifies a specific region of a DNA strand (the DNA target). Most PCR methods amplify DNA fragments of between 0.1 and 10 kilo base pairs (kbp), although some techniques allow for amplification of fragments up to 40 kbp in size. The amount of amplified product is determined by the available substrates in the reaction, which become limiting as the reaction progresses.

A basic PCR set-up requires several components and reagents, including:

- a *DNA template* that contains the DNA target region to amplify
- a *DNA polymerase*, an enzyme that polymerizes new DNA strands; heat-resistant Taq polymerase is especially common, as it is more likely to remain intact during the high-temperature DNA denaturation process
- two DNA *primers* that are complementary to the 3’ (three prime) ends of each of the sense and anti-sense strands of the DNA target (DNA polymerase can only bind to and elongate from a double-stranded region of DNA; without primers there is no double-stranded initiation site at which the polymerase can bind); specific primers that are complementary to the DNA target region are selected beforehand, and are often custom-made in a laboratory or purchased from commercial biochemical suppliers
- *deoxynucleoside triphosphates*, or dNTPs (sometimes called "deoxynucleotide triphosphates"; nucleotides containing triphosphate groups), the building blocks from which the DNA polymerase synthesizes a new DNA strand
- a *buffer solution* providing a suitable chemical environment for optimum activity and stability of the DNA polymerase
- *bivalent cations*, typically magnesium (Mg) or manganese (Mn) ions; Mg$^{2+}$ is the most common, but Mn$^{2+}$ can be used for PCR-mediated DNA mutagenesis, as a higher Mn$^{2+}$ concentration increases the error rate during DNA synthesis
- *monovalent cations*, typically potassium (K) ions

The reaction is commonly carried out in a volume of 10–200 μl in small reaction tubes (0.2–0.5 ml volumes) in a thermal cycler. The thermal cycler heats and cools the reaction tubes to achieve the temperatures required at each step of the reaction (see below). Many modern thermal cyclers make use of the Peltier effect, which permits both heating and cooling of the block holding the PCR tubes simply by reversing the electric current. Thin-walled reaction tubes
permit favorable thermal conductivity to allow for rapid thermal equilibration. Most thermal cyclers have heated lids to prevent condensation at the top of the reaction tube. Older thermal cyclers lacking a heated lid require a layer of oil on top of the reaction mixture or a ball of wax inside the tube.

### 3.2.2.1. Procedure

Typically, PCR consists of a series of 20–40 repeated temperature changes, called cycles, with each cycle commonly consisting of two or three discrete temperature steps. The cycling is often preceded by a single temperature step at a very high temperature (>90 °C (194 °F)), and followed by one hold at the end for final product extension or brief storage. The temperatures used and the length of time they are applied in each cycle depend on a variety of parameters, including the enzyme used for DNA synthesis, the concentration of bivalent ions and dNTPs in the reaction, and the melting temperature ($T_m$) of the primers. The individual steps common to most PCR methods are as follows:

**Initialization**: This step is only required for DNA polymerases that require heat activation by hot-start PCR. It consists of heating the reaction chamber to a temperature of 94–96 °C (201–205 °F), or 98 °C (208 °F) if extremely thermostable polymerases are used, which is then held for 1–10 minutes.

**Denaturation**: This step is the first regular cycling event and consists of heating the reaction chamber to 94–98 °C (201–208 °F) for 20–30 seconds. This causes DNA melting, or denaturation, of the double-stranded DNA template by breaking the hydrogen bonds between complementary bases, yielding two single-stranded DNA molecules.

**Annealing**: In the next step, the reaction temperature is lowered to 50–65 °C (122–149 °F) for 20–40 seconds, allowing annealing of the primers to each of the single-stranded DNA templates. Two different primers are typically included in the reaction mixture: one for each of the two single-stranded complements containing the target region. The primers are single-stranded sequences themselves, but are much shorter than the length of the target region, complementing only very short sequences at the 3' end of each strand. It is critical to determine a proper temperature for the annealing step because efficiency and specificity are strongly affected by the
annealing temperature. This temperature must be low enough to allow for hybridization of the primer to the strand, but high enough for the hybridization to be specific, i.e., the primer should bind only to a perfectly complementary part of the strand, and nowhere else. If the temperature is too low, the primer may bind imperfectly. If it is too high, the primer may not bind at all. A typical annealing temperature is about 3–5 °C below the $T_m$ of the primers used. Stable hydrogen bonds between complementary bases are formed only when the primer sequence very closely matches the template sequence. During this step, the polymerase binds to the primer-template hybrid and begins DNA formation.

**Extension/elongation:** The temperature at this step depends on the DNA polymerase used; the optimum activity temperature for Taq polymerase is approximately 75–80 °C (167–176 °F), though a temperature of 72 °C (162 °F) is commonly used with this enzyme. In this step, the DNA polymerase synthesizes a new DNA strand complementary to the DNA template strand by adding free dNTPs from the reaction mixture that are complementary to the template in the 5'-to-3' direction, condensing the 5'-phosphate group of the dNTPs with the 3'-hydroxy group at the end of the nascent (elongating) DNA strand. The precise time required for elongation depends both on the DNA polymerase used and on the length of the DNA target region to amplify. As a rule of thumb, at their optimal temperature, most DNA polymerases polymerize a thousand bases per minute. Under optimal conditions (i.e., if there are no limitations due to limiting substrates or reagents), at each extension/elongation step, the number of DNA target sequences is doubled. With each successive cycle, the original template strands plus all newly generated strands become template strands for the next round of elongation, leading to exponential (geometric) amplification of the specific DNA target region. The processes of denaturation, annealing and elongation constitute a single cycle. Multiple cycles are required to amplify the DNA target to millions of copies. The formula used to calculate the number of DNA copies formed after a given number of cycles is $2^n$, where $n$ is the number of cycles. Thus, a reaction set for 30 cycle’s results in $2^{30}$, or 1073741824, copies of the original double-stranded DNA target region.

**Final elongation:** This single step is optional, but is performed at a temperature of 70–74 °C (158–165 °F) (the temperature range required for optimal activity of most polymerases used in PCR) for 5–15 minutes after the last PCR cycle to ensure that any remaining single-stranded DNA is fully elongated.
**Final hold:** The final step cools the reaction chamber to 4–15 °C (39–59 °F) for an indefinite time, and may be employed for short-term storage of the PCR products.

Ethidium bromide-stained PCR products after gel electrophoresis, two sets of primers were used to amplify a target sequence from three different tissue samples. No amplification is present in sample #1; DNA bands in sample #2 and #3 indicate successful amplification of the target sequence. The gel also shows a positive control, and a DNA ladder containing DNA fragments of defined length for sizing the bands in the experimental PCRs.

To check whether the PCR successfully generated the anticipated DNA target region (also sometimes referred to as the amplimer or amplicon), agarose gel electrophoresis may be employed for size separation of the PCR products. The size(s) of PCR products is determined by comparison with a DNA ladder, a molecular weight marker which contains DNA fragments of known size run on the gel alongside the PCR products.
Stages

As with other chemical reactions, the reaction rate and efficiency of PCR are affected by limiting factors. Thus, the entire PCR process can further be divided into three stages based on reaction progress:

1. The DNA double helix is melted apart at T> 90°C and its strands separate.
2. The temperature is decreased to slightly below the T_m of both the primers being used. Both primers bind to the available strands. These primers are supplied in excess to ensure that the strands do not only come back and reanneal to one another.
3. Polymerization (extension) occurs via DNA Polymerase in the 5' to 3' direction on each strand.
4. Incorporated additional nucleotides give rise to new strands that extend past the sequence of interest.
5. The previously polymerized strands act as template for the other primer (if forward primer bound first, reverse primer now binds and vice versa).
6. Polymerization occurs via DNA Polymerase in the 5' to 3' direction on each strand, this time ending at the end of the sequence of interest.
7. Incorporated additional nucleotides give rise to new strands that only encode the sequence of interest.
8. The synthesized strands encoding the sequence of interest anneal to one another to form the end product.
3.2.3. Optimization

In practice, PCR can fail for various reasons, in part due to its sensitivity to contamination causing amplification of spurious DNA products. Because of this, a number of techniques and procedures have been developed for optimizing PCR conditions. Contamination with extraneous DNA is addressed with lab protocols and procedures that separate pre-PCR mixtures from potential DNA contaminants. This usually involves spatial separation of PCR-setup areas from areas for analysis or purification of PCR products, use of disposable plastic ware, and thoroughly cleaning the work surface between reaction setups. Primer-design techniques are important in improving PCR product yield and in avoiding the formation of spurious products, and the usage of alternate buffer components or polymerase enzymes can help with amplification of long or otherwise problematic regions of DNA. Addition of reagents, such as formamide, in buffer systems may increase the specificity and yield of PCR. Computer simulations of theoretical PCR results (Electronic PCR) may be performed to assist in primer design.

3.2.4. Applications

3.2.4.1. Selective DNA isolation

PCR allows isolation of DNA fragments from genomic DNA by selective amplification of a specific region of DNA. This use of PCR augments many ways, such as generating hybridization probes for Southern or northern hybridization and DNA cloning, which require larger amounts of DNA, representing a specific DNA region. PCR supplies these techniques with high amounts of pure DNA, enabling analysis of DNA samples even from very small amounts of starting material. Other applications of PCR include DNA sequencing to determine unknown PCR-amplified sequences in which one of the amplification primers may be used in Sanger
sequencing, isolation of a DNA sequence to expedite recombinant DNA technologies involving the insertion of a DNA sequence into a plasmid, phage, or cosmid (depending on size) or the genetic material of another organism. Bacterial colonies (such as E. coli) can be rapidly screened by PCR for correct DNA vector constructs. PCR may also be used for genetic fingerprinting; a forensic technique used to identify a person or organism by comparing experimental DNAs through different PCR-based methods. Some PCR 'fingerprints' methods have high discriminative power and can be used to identify genetic relationships between individuals, such as parent-child or between siblings, and are used in paternity testing. This technique may also be used to determine evolutionary relationships among organisms when certain molecular clocks are used (i.e., the 16S rRNA and recA genes of microorganisms).

Electrophoresis of PCR-amplified DNA fragments. (1) Father. (2) Child. (3) Mother. The child has inherited some, but not the entire fingerprint of each of its parents, giving it a new, unique fingerprint.

### 3.2.4.2. Amplification and quantification of DNA

Because PCR amplifies the regions of DNA that it targets, PCR can be used to analyze extremely small amounts of sample. This is often critical for forensic analysis, when only a trace amount of DNA is available as evidence. PCR may also be used in the analysis of ancient DNA that is tens of thousands of years old. These PCR-based techniques have been successfully used on animals, such as a forty-thousand-year-old mammoth, and also on human DNA, in applications ranging from the analysis of Egyptian mummies to the identification of a Russian tsar and the body of English king Richard III. Quantitative PCR (qPCR) methods allow the estimation of the amount of a given sequence present in a sample—a technique often applied to quantitatively determine levels of gene expression. Quantitative PCR is an established tool for DNA quantification that measures the accumulation of DNA product after each round of PCR amplification.

qPCR allows the quantification and detection of a specific DNA sequence in real time since it measures concentration while the synthesis process is taking place. There are two methods for simultaneous detection and quantification. The first method consists of using fluorescent dyes that are retained nonspecifically in between the double strands. The second method involves probes that code for specific sequences and are fluorescently labeled. Detection of DNA using these methods can only be seen after the hybridization of probes with its complementary DNA.
takes place. An interesting technique combination is real-time PCR and reverse transcription (RT-qPCR). This sophisticated technique allows for the quantification of a small quantity of RNA. Through this combined technique, mRNA is converted to cDNA, which is further quantified using qPCR. This technique lowers the possibility of error at the end point of PCR, increasing chances for detection of genes associated with genetic diseases such as cancer. Laboratories use RT-qPCR for the purpose of sensitively measuring gene regulation.

3.2.4.3. Medical applications

After the completion of sequencing of the first genome in 2000, the Human Genome Project, PCR has been applied to a large number of medical procedures:

The first application of PCR was used for genetic testing, where a sample of DNA was analyzed for the presence of genetic disease mutations. Prospective parents can be tested for being genetic carriers, or their children might be tested for actually being affected by a disease. DNA samples for prenatal testing can be obtained by amniocentesis, chorionic villus sampling, or even by the analysis of rare fetal cells circulating in the mother's bloodstream. PCR analysis is also essential to preimplantation genetic diagnosis, where individual cells of a developing embryo are tested for mutations.

PCR can also be used as part of a sensitive test for tissue typing, vital to organ transplantation. As of 2008, there is even a proposal to replace the traditional antibody-based tests for blood type with PCR-based tests.

Many forms of cancer involve alterations to oncogenes. By using PCR-based tests to study these mutations, therapy regimens can sometimes be individually customized to a patient. PCR permits early diagnosis of malignant diseases such as leukemia and lymphomas, which is currently the highest-developed in cancer research and is already being used routinely. PCR assays can be performed directly on genomic DNA samples to detect translocation-specific malignant cells at a sensitivity that is at least 10,000 fold higher than that of other methods. PCR is very useful in the medical field since it allows for the isolation and amplification of tumor suppressors. Quantitative PCR for example, can be used to quantify and analyze single cells, as well as recognize DNA, mRNA and protein confirmations and combinations.
3.2.4.4. Infectious disease applications

PCR allows for rapid and highly specific diagnosis of infectious diseases, including those caused by bacteria or viruses. PCR also permits identification of non-cultivatable or slow-growing microorganisms such as mycobacteria, anaerobic bacteria, or viruses from tissue culture assays and animal models. The basis for PCR diagnostic applications in microbiology is the detection of infectious agents and the discrimination of non-pathogenic from pathogenic strains by virtue of specific genes.

Characterization and detection of infectious disease organisms have been revolutionized by PCR in the following ways:

The human immunodeficiency virus (or HIV), is a difficult target to find and eradicate. The earliest tests for infection relied on the presence of antibodies to the virus circulating in the bloodstream. However, antibodies don't appear until many weeks after infection, maternal antibodies mask the infection of a newborn, and therapeutic agents to fight the infection don't affect the antibodies. PCR tests have been developed that can detect as little as one viral genome among the DNA of over 50,000 host cells. Infections can be detected earlier, donated blood can be screened directly for the virus, newborns can be immediately tested for infection, and the effects of antiviral treatments can be quantified.

Some disease organisms, such as that for tuberculosis, are difficult to sample from patients and slow to be grown in the laboratory. PCR-based tests have allowed detection of small numbers of disease organisms (both live and dead), in convenient samples. Detailed genetic analysis can also be used to detect antibiotic resistance, allowing immediate and effective therapy. The effects of therapy can also be immediately evaluated.

The spread of a disease organism through populations of domestic or wild animals can be monitored by PCR testing. In many cases, the appearance of new virulent sub-types can be detected and monitored. The sub-types of an organism that were responsible for earlier epidemics can also be determined by PCR analysis.
Viral DNA can be detected by PCR. The primers used must be specific to the targeted sequences in the DNA of a virus, and PCR can be used for diagnostic analyses or DNA sequencing of the viral genome. The high sensitivity of PCR permits virus detection soon after infection and even before the onset of disease. Such early detection may give physicians a significant lead time in treatment. The amount of virus ("viral load") in a patient can also be quantified by PCR-based DNA quantitation techniques.

3.2.4.5. Forensic applications

The development of PCR-based genetic (or DNA) fingerprinting protocols has seen widespread application in forensics:

In its most discriminating form, genetic fingerprinting can uniquely discriminate any one person from the entire population of the world. Minute samples of DNA can be isolated from a crime scene, and compared to that from suspects, or from a DNA database of earlier evidence or convicts. Simpler versions of these tests are often used to rapidly rule out suspects during a criminal investigation. Evidence from decades-old crimes can be tested, confirming or exonerating the people originally convicted.

Forensic DNA typing has been an effective way of identifying or exonerating criminal suspects due to analysis of evidence discovered at a crime scene. The human genome has many repetitive regions that can be found within gene sequences or in non-coding regions of the genome. Specifically, up to 40% of human DNA is repetitive. There are two distinct categories for these repetitive, non-coding regions in the genome. The first category is called variable number tandem repeats (VNTR), which are 10-100 base pairs long and the second category is called short tandem repeats (STR) and these consist of repeated 2-10 base pair sections. PCR is used to amplify several well-known VNTRs and STRs using primers that flank each of the repetitive regions. The sizes of the fragments obtained from any individual for each of the STRs will indicate which alleles are present. By analyzing several STRs for an individual, a set of alleles for each person will be found that statistically is likely to be unique. Researchers have identified the complete sequence of the human genome. This sequence can be easily accessed through the NCBI website and is used in many real-life applications. For example, the FBI has compiled a
set of DNA marker sites used for identification, and these are called the Combined DNA Index System (CODIS) DNA database. Using this database enables statistical analysis to be used to determine the probability that a DNA sample will match. PCR is a very powerful and significant analytical tool to use for forensic DNA typing because researchers only need a very small amount of the target DNA to be used for analysis. For example, a single human hair with attached hair follicle has enough DNA to conduct the analysis. Similarly, a few sperm, skin samples from under the fingernails, or a small amount of blood can provide enough DNA for conclusive analysis.

Less discriminating forms of DNA fingerprinting can help in DNA paternity testing, where an individual is matched with their close relatives. DNA from unidentified human remains can be tested, and compared with that from possible parents, siblings, or children. Similar testing can be used to confirm the biological parents of an adopted (or kidnapped) child. The actual biological father of a newborn can also be confirmed (or ruled out).

3.2.4.6. Research applications

PCR has been applied to many areas of research in molecular genetics:

PCR allows rapid production of short pieces of DNA, even when not more than the sequence of the two primers is known. This ability of PCR augments many methods, such as generating hybridization probes for Southern or northern blot hybridization. PCR supplies these techniques with large amounts of pure DNA, sometimes as a single strand, enabling analysis even from very small amounts of starting material.

The task of DNA sequencing can also be assisted by PCR. Known segments of DNA can easily be produced from a patient with a genetic disease mutation. Modifications to the amplification technique can extract segments from a completely unknown genome, or can generate just a single strand of an area of interest.

PCR has numerous applications to the more traditional process of DNA cloning. It can extract segments for insertion into a vector from a larger genome, which may be only available in small quantities. Using a single set of 'vector primers', it can also analyze or extract fragments that
have already been inserted into vectors. Some alterations to the PCR protocol can generate mutations (general or site-directed) of an inserted fragment.

Sequence - tagged sites is a process where PCR is used as an indicator that a particular segment of a genome is present in a particular clone. The Human Genome Project found this application vital to mapping the cosmid clones they were sequencing, and to coordinating the results from different laboratories.

An exciting application of PCR is the phylogenic analysis of DNA from ancient sources, such as that found in the recovered bones of Neanderthals, or from frozen tissues of mammoths. In some cases the highly degraded DNA from these sources might be reassembled during the early stages of amplification.

A common application of PCR is the study of patterns of gene expression. Tissues (or even individual cells) can be analyzed at different stages to see which genes have become active, or which have been switched off. This application can also use quantitative PCR to quantitate the actual levels of expression.

The ability of PCR to simultaneously amplify several loci from individual sperm has greatly enhanced the more traditional task of genetic mapping by studying chromosomal crossovers after meiosis. Rare crossover events between very close loci have been directly observed by analyzing thousands of individual sperms. Similarly, unusual deletions, insertions, translocations, or inversions can be analyzed, all without having to wait (or pay) for the long and laborious processes of fertilization, embryogenesis, etc.

3.2.5. Advantages

PCR has a number of advantages. It is fairly simple to understand and to use, and produces results rapidly. The technique is highly sensitive with the potential to produce millions to billions of copies of a specific product for sequencing, cloning, and analysis. qRT - PCR shares the same advantages as the PCR, with an added advantage of quantification of the synthesized product. Therefore, it has its uses to analyze alterations of gene expression levels in tumors, microbes, or other disease states.
3.2.6. Limitations

One major limitation of PCR is that prior information about the target sequence is necessary in order to generate the primers that will allow its selective amplification. This means that, typically, PCR users must know the precise sequence(s) upstream of the target region on each of the two single-stranded templates in order to ensure that the DNA polymerase properly binds to the primer-template hybrids and subsequently generates the entire target region during DNA synthesis. Like all enzymes, DNA polymerases are also prone to error, which in turn causes mutations in the PCR fragments that are generated.

3.2.7. Variations

*Allele-specific PCR*: a diagnostic or cloning technique based on single-nucleotide variations (SNVs not to be confused with SNPs) (single-base differences in a patient). It requires prior knowledge of a DNA sequence, including differences between alleles, and uses primers whose 3’ ends encompass the SNV (base pair buffer around SNV usually incorporated). PCR amplification under stringent conditions is much less efficient in the presence of a mismatch between template and primer, so successful amplification with an SNP-specific primer signals presence of the specific SNP in a sequence.

*Assembly PCR* or *Polymerase Cycling Assembly (PCA)*: artificial synthesis of long DNA sequences by performing PCR on a pool of long oligonucleotides with short overlapping segments. The oligonucleotides alternate between sense and antisense directions and the overlapping segments determine the order of the PCR fragments, thereby selectively producing the final long DNA product.

*Asymmetric PCR*: preferentially amplifies one DNA strand in a double-stranded DNA template. It is used in sequencing and hybridization probing where amplification of only one of the two complementary strands is required. PCR is carried out as usual, but with a great excess of the primer for the strand targeted for amplification. Because of the slow (arithmetic) amplification later in the reaction after the limiting primer has been used up, extra cycles of PCR are required. A recent modification on this process, known as *Linear-After-The-Exponential-PCR* (LATE-
PCR), uses a limiting primer with a higher melting temperature (Tm) than the excess primer to maintain reaction efficiency as the limiting primer concentration decreases mid-reaction.

**Convective PCR**: a pseudo-isothermal way of performing PCR. Instead of repeatedly heating and cooling the PCR mixture, the solution is subjected to a thermal gradient. The resulting thermal instability driven convective flow automatically shuffles the PCR reagents from the hot and cold regions repeatedly enabling PCR. Parameters such as thermal boundary conditions and geometry of the PCR enclosure can be optimized to yield robust and rapid PCR by harnessing the emergence of chaotic flow fields. Such convective flow PCR setup significantly reduces device power requirement and operation time.

**Dial-out PCR**: a highly parallel method for retrieving accurate DNA molecules for gene synthesis. A complex library of DNA molecules is modified with unique flanking tags before massively parallel sequencing. Tag-directed primers then enable the retrieval of molecules with desired sequences by PCR.

**Digital PCR (dPCR)**: used to measure the quantity of a target DNA sequence in a DNA sample. The DNA sample is highly diluted so that after running many PCRs in parallel, some of them do not receive a single molecule of the target DNA. The target DNA concentration is calculated using the proportion of negative outcomes. Hence the name 'digital PCR'

**Helicase-dependent amplification**: similar to traditional PCR, but uses a constant temperature rather than cycling through denaturation and annealing/extension cycles. DNA helicase, an enzyme that unwinds DNA, is used in place of thermal denaturation.

**Hot start PCR**: a technique that reduces non-specific amplification during the initial set up stages of the PCR. It may be performed manually by heating the reaction components to the denaturation temperature (e.g., 95 °C) before adding the polymerase. Specialized enzyme systems have been developed that inhibit the polymerase's activity at ambient temperature, either by the binding of an antibody or by the presence of covalently bound inhibitors that dissociate only after a high-temperature activation step. Hot-start/cold-finish PCR is achieved with new hybrid polymerases that are inactive at ambient temperature and are instantly activated at elongation temperature.
**In silico PCR** (digital PCR, virtual PCR, electronic PCR, e-PCR) refers to computational tools used to calculate theoretical polymerase chain reaction results using a given set of primers (probes) to amplify DNA sequences from a sequenced genome or transcriptome. In silico PCR was proposed as an educational tool for molecular biology.

**Intersequence-specific PCR** (ISSR): a PCR method for DNA fingerprinting that amplifies regions between simple sequence repeats to produce a unique fingerprint of amplified fragment lengths.

**Inverse PCR**: is commonly used to identify the flanking sequences around genomic inserts. It involves a series of DNA digestions and self ligation, resulting in known sequences at either end of the unknown sequence.

**Ligation-mediated PCR**: uses small DNA linkers ligated to the DNA of interest and multiple primers annealing to the DNA linkers; it has been used for DNA sequencing, genome walking, and DNA footprinting.

**Methylation-specific PCR** (MSP): developed by Stephen Baylin and Jim Herman at the Johns Hopkins School of Medicine, and is used to detect methylation of CpG islands in genomic DNA. DNA is first treated with sodium bisulfite, which converts unmethylated cytosine bases to uracil, which is recognized by PCR primers as thymine. Two PCRs are then carried out on the modified DNA, using primer sets identical except at any CpG islands within the primer sequences. At these points, one primer set recognizes DNA with cytosines to amplify methylated DNA, and one set recognizes DNA with uracil or thymine to amplify unmethylated DNA. MSP using qPCR can also be performed to obtain quantitative rather than qualitative information about methylation.

**Miniprimer PCR**: uses a thermostable polymerase (S-Tbr) that can extend from short primers ("smalligos") as short as 9 or 10 nucleotides. This method permits PCR targeting to smaller primer binding regions, and is used to amplify conserved DNA sequences, such as the 16S (or eukaryotic 18S) rRNA gene.
Multiplex ligation-dependent probe amplification (MLPA): permits amplifying multiple targets with a single primer pair, thus avoiding the resolution limitations of multiplex PCR.

Multiplex-PCR: consists of multiple primer sets within a single PCR mixture to produce amplicons of varying sizes that are specific to different DNA sequences. By targeting multiple genes at once, additional information may be gained from a single test-run that otherwise would require several times the reagents and more time to perform. Annealing temperatures for each of the primer sets must be optimized to work correctly within a single reaction, and amplicon sizes. That is, their base pair length should be different enough to form distinct bands when visualized by gel electrophoresis.

Nanoparticle-Assisted PCR (nanoPCR): In recent years, it has been reported that some nanoparticles (NPs) can enhance the efficiency of PCR (thus being called nanoPCR), and some even perform better than the original PCR enhancers. It was also found that quantum dots (QDs) can improve PCR specificity and efficiency. Single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs) are efficient in enhancing the amplification of long PCR. Carbon nanopowder (CNP) was reported be able to improve the efficiency of repeated PCR and long PCR. ZnO, TiO2, and Ag NPs were also found to increase PCR yield. Importantly, already known data has indicated that non-metallic NPs retained acceptable amplification fidelity. Given that many NPs are capable of enhancing PCR efficiency, it is clear that there is likely to be great potential for nanoPCR technology improvements and product development.

Nested PCR: increases the specificity of DNA amplification, by reducing background due to non-specific amplification of DNA. Two sets of primers are used in two successive PCRs. In the first reaction, one pair of primers is used to generate DNA products, which besides the intended target, may still consist of non-specifically amplified DNA fragments. The product(s) are then used in a second PCR with a set of primers whose binding sites are completely or partially different from and located 3' of each of the primers used in the first reaction. Nested PCR is often more successful in specifically amplifying long DNA fragments than conventional PCR, but it requires more detailed knowledge of the target sequences.
Overlap-extension PCR or Splicing by overlap extension (SOEing): a genetic engineering technique that is used to splice together two or more DNA fragments that contain complementary sequences. It is used to join DNA pieces containing genes, regulatory sequences, or mutations; the technique enables creation of specific and long DNA constructs. It can also introduce deletions, insertions or point mutations into a DNA sequence.

PAN-AC: uses isothermal conditions for amplification, and may be used in living cells.

Quantitative PCR (qPCR): used to measure the quantity of a target sequence (commonly in real-time). It quantitatively measures starting amounts of DNA, cDNA, or RNA. Quantitative PCR is commonly used to determine whether a DNA sequence is present in a sample and the number of its copies in the sample. Quantitative PCR has a very high degree of precision. Quantitative PCR methods use fluorescent dyes, such as Sybr Green, EvaGreen or fluorophore-containing DNA probes, such as TaqMan, to measure the amount of amplified product in real time. It is also sometimes abbreviated to RT-PCR (real-time PCR) but this abbreviation should be used only for reverse transcription PCR. qPCR is the appropriate contractions for quantitative PCR (real-time PCR).

Reverse Transcription PCR (RT-PCR): for amplifying DNA from RNA. Reverse transcriptase reverse transcribes RNA into cDNA, which is then amplified by PCR. RT-PCR is widely used in expression profiling, to determine the expression of a gene or to identify the sequence of an RNA transcript, including transcription start and termination sites. If the genomic DNA sequence of a gene is known, RT-PCR can be used to map the location of exons and introns in the gene. The 5' end of a gene (corresponding to the transcription start site) is typically identified by RACE-PCR (Rapid Amplification of cDNA Ends).

RNase H-dependent PCR (rhPCR): a modification of PCR that utilizes primers with a 3’ extension block that can be removed by a thermostable RNase HII enzyme. This system reduces primer-dimers and allows for multiplexed reactions to be performed with higher numbers of primers.

Single Specific Primer-PCR (SSP-PCR): allows the amplification of double-stranded DNA even when the sequence information is available at one end only. This method permits amplification
of genes for which only partial sequence information is available, and allows unidirectional genome walking from known into unknown regions of the chromosome.

**Solid Phase PCR**: encompasses multiple meanings, including Polony Amplification (where PCR colonies are derived in a gel matrix, for example), Bridge PCR (primers are covalently linked to a solid-support surface), conventional Solid Phase PCR (where Asymmetric PCR is applied in the presence of solid support bearing primer with sequence matching one of the aqueous primers) and Enhanced Solid Phase PCR (where conventional Solid Phase PCR can be improved by employing high Tm and nested solid support primer with optional application of a thermal 'step' to favour solid support priming).

**Suicide PCR**: typically used in paleogenetics or other studies where avoiding false positives and ensuring the specificity of the amplified fragment is the highest priority. It was originally described in a study to verify the presence of the microbe Yersinia pestis in dental samples obtained from 14th Century graves of people supposedly killed by plague during the medieval Black Death epidemic. The method prescribes the use of any primer combination only once in a PCR (hence the term "suicide"), which should never have been used in any positive control PCR reaction, and the primers should always target a genomic region never amplified before in the lab using this or any other set of primers. This ensures that no contaminating DNA from previous PCR reactions is present in the lab, which could otherwise generate false positives.

**Thermal asymmetric interlaced PCR (TAIL-PCR)**: for isolation of an unknown sequence flanking a known sequence. Within the known sequence, TAIL-PCR uses a nested pair of primers with differing annealing temperatures; a degenerate primer is used to amplify in the other direction from the unknown sequence.

**Touchdown PCR (Step-down PCR)**: a variant of PCR that aims to reduce nonspecific background by gradually lowering the annealing temperature as PCR cycling progresses. The annealing temperature at the initial cycles is usually a few degrees (3–5 °C) above the Tm of the primers used, while at the later cycles, it is a few degrees (3–5 °C) below the primer Tm. The higher temperatures give greater specificity for primer binding, and the lower temperatures permit more efficient amplification from the specific products formed during the initial cycles.
Universal Fast Walking: for genome walking and genetic fingerprinting using a more specific 'two-sided' PCR than conventional 'one-sided' approaches (using only one gene-specific primer and one general primer which can lead to artefactual 'noise') by virtue of a mechanism involving lariat structure formation. Streamlined derivatives of UFW are LaNe RAGE (lariat-dependent nested PCR for rapid amplification of genomic DNA ends), 5'RACE LaNe and 3'RACE LaNe.

3.2.8. History

A 1971 paper in the Journal of Molecular Biology by Kjell Kleppe (no) and co-workers in the laboratory of H. Gobind Khorana first described a method using an enzymatic assay to replicate a short DNA template with primers in vitro. However, this early manifestation of the basic PCR principle did not receive much attention at the time, and the invention of the polymerase chain reaction in 1983 is generally credited to Kary Mullis.

"Baby Blue", a 1986 prototype machine for doing PCR

When Mullis developed the PCR in 1983, he was working in Emeryville, California for Cetus Corporation, one of the first biotechnology companies. There, he was responsible for synthesizing short chains of DNA. Mullis has written that he conceived of PCR while cruising along the Pacific Coast Highway one night in his car. He was playing in his mind with a new way of analyzing changes (mutations) in DNA when he realized that he had instead invented a method of amplifying any DNA region through repeated cycles of duplication driven by DNA polymerase. In Scientific American, Mullis summarized the procedure: "Beginning with a single molecule of the genetic material DNA, the PCR can generate 100 billion similar molecules in an afternoon. The reaction is easy to execute. It requires no more than a test tube, a few simple reagents, and a source of heat." In 1988, DNA fingerprinting first became used for paternity testing in 1988. Mullis was awarded the Nobel Prize in Chemistry in 1993 for his invention,
seven years after he and his colleagues at Cetus first put his proposal to practice. However, some controversies have remained about the intellectual and practical contributions of other scientists to Mullis' work, and whether he had been the sole inventor of the PCR principle (see below). At the core of the PCR method is the use of a suitable DNA polymerase able to withstand the high temperatures of >90 °C (194 °F) required for separation of the two DNA strands in the DNA double helix after each replication cycle. The DNA polymerases initially employed for in vitro experiments presaging PCR were unable to withstand these high temperatures. So the early procedures for DNA replication were very inefficient and time-consuming, and required large amounts of DNA polymerase and continuous handling throughout the process. The discovery in 1976 of Taq polymerase a DNA polymerase purified from the thermophilic bacterium, *Thermus aquaticus*, which naturally lives in hot (50 to 80 °C (122 to 176 °F)) environments such as hot springs paved the way for dramatic improvements of the PCR method. The DNA polymerase isolated from *T. aquaticus* is stable at high temperatures remaining active even after DNA denaturation, thus obviating the need to add new DNA polymerase after each cycle. This allowed an automated thermocycler-based process for DNA amplification.

### 3.2.8.1. Patent disputes

The PCR technique was patented by Kary Mullis and assigned to Cetus Corporation, where Mullis worked when he invented the technique in 1983. The *Taq* polymerase enzyme was also covered by patents. There have been several high-profile lawsuits related to the technique, including an unsuccessful lawsuit brought by DuPont. The pharmaceutical company Hoffmann-La Roche purchased the rights to the patents in 1992 and currently holds those that are still protected. A related patent battle over the Taq polymerase enzyme is still ongoing in several jurisdictions around the world between Roche and Promega. The legal arguments have extended beyond the lives of the original PCR and Taq polymerase patents, which expired on March 28, 2005.

### 3.3. Point-of-care-diagnostic devices

Point-of-care testing (POCT) or bedside testing is defined as medical diagnostic testing at or near the point of care that is, at the time and place of patient care. This contrasts with the historical
pattern in which testing was wholly or mostly confined to the medical laboratory, which entailed sending off specimens away from the point of care and then waiting hours or days to learn the results, during which time care must continue without the desired information. Point-of-care tests are simple medical tests that can be performed at the bedside. In many cases the simplicity was not achievable until technology developed not only to make a test possible at all but then also to mask its complexity. For example, various kinds of urine test strips have been available for decades, but portable ultrasonography did not reach the stage of being advanced, affordable, and widespread until the 2000s and 2010s. Today portable US are often viewed as a "simple" test, but there was nothing simple about it until the more complex technology was available. Similarly, pulse oximetry can test arterial oxygen saturation in a quick, simple, noninvasive, affordable way today, but in earlier eras this required an intraarterial needle puncture and a laboratory test; and rapid diagnostic tests such as malaria antigen detection tests rely on a state of the art in immunology that did not exist until recent decades. Thus, over decades, testing continues to move toward the point of care more than it formerly had been. A recent survey in five countries (Australia, Belgium, the Netherlands, the UK and the US) indicates that general practitioners / family doctors would like to use more POCTs.

The driving notion behind POCT is to bring the test conveniently and immediately to the patient. This increases the likelihood that the patient, physician, and care team will receive the results quicker, which allows for better immediate clinical management decisions to be made. POCT includes: blood glucose testing, blood gas and electrolytes analysis, rapid coagulation testing (PT/INR, Alere, Micr0visk Ltd), rapid cardiac markers diagnostics (TRIAGE, Alere), drugs of abuse screening, urine strips testing, pregnancy testing, fecal occult blood analysis, food pathogens screening, hemoglobin diagnostics (HemoCue), infectious disease testing and cholesterol screening. POCT is often accomplished through the use of transportable, portable, and handheld instruments (e.g., blood glucose meter, nerve conduction study device) and test kits (e.g., CRP, HBA1C, Homocysteine, HIV salivary assay, etc.). Small bench analyzers or fixed equipment can also be used when a handheld device is not available the goal is to collect the specimen and obtain the results in a very short period of time at or near the location of the patient so that the treatment plan can be adjusted as necessary before the patient leaves. Cheaper, faster, and smarter POCT devices have increased the use of POCT approaches by making it cost-
effective for many diseases, such as diabetes, carpal tunnel syndrome (CTS) and acute coronary syndrome. Additionally, it is very desirable to measure various analytes simultaneously in the same specimen, allowing a rapid, low-cost, and reliable quantification. Therefore, multiplexed point-of-care testing (xPOCT) has become more important for medical diagnostics in the last decade.

3.3.1. Technology

Many point-of-care test systems are realized as easy-to-use membrane-based test strips, often enclosed by a plastic test cassette. This concept often is realized in test systems for detecting pathogens. Very recently such test systems for rheumatology diagnostics have been developed, too. These tests require only a single drop of whole blood, urine or saliva, and they can be performed and interpreted by any general physician within minutes.

3.3.2. Benefits

Major benefits are obtained when the output of a POCT device is made available immediately within an electronic medical record. Results can be shared instantaneously with all members of the medical team through the software interface enhancing communication by decreasing turnaround time (TAT). A reduction in morbidity and mortality has been associated with goal-directed therapy (GDT) techniques when used in conjunction with POCT and the electronic medical record. POCT has become established worldwide and finds vital roles in public health. Many monographs in the Thai and Indonesian languages emphasize POCT as the normal standard of care in disaster situation. Potential operational benefits of POCT: more rapid decision making and triage, reduce operating times, reduce high-dependency, postoperative care time, reduce emergency room time, reduce number of outpatient clinic visits, reduce number of hospital beds required, ensure optimal use of professional time.

3.3.3. Multiplexed point-of-care testing

Multiplexed point-of-care testing (xPOCT) is the simultaneous on-site quantification of various analytes from a single sample (e.g., blood, plasma or urine). Derived from emerging applications
in resource-limited settings, (e.g., in the developing countries, in doctor's practices, or at home) xPOCT has recently become more important for in vitro diagnostics.

### 3.3.4. Requirements

xPOCT devices has to completely fulfill the following demands:

- Low sample consumption (e.g., blood from a finger prick) or the ability to measure in noninvasive samples (e.g., saliva, urine or exhaled breath condensate)
- Fast sample-to-result times enabling an immediate treatment
- Long shelf life with extended reagent storage
- Comparable test results with central laboratory findings ensuring international quality standards (ISO 15189)
- Automatic or facile system operation with minimized user intervention
- Cheap and portable readout systems (e.g., handheld readers) along with disposable test strips or cartridges fulfilling the in vitro diagnostics guideline (EU Directives or FDA regulations).

Especially for the resource-poor settings, equipment-free or smartphone-based devices are very advantageous. Besides, an ideal xPOCT device should be capable of testing various kinds of substances, including proteins, drugs, RNAs and cells, at the same time.

### 3.3.5. Current multiplexing technologies

Nowadays, multianalyte detection is mostly achieved through three different approaches: (1) Regional separation employing distinct sections of a channel network or array of electrodes, (2) Spatial separation of detection sites with the help of various wells or spots, (3) Application of multiple labels such as enzymes, redox molecules, beads, and dyes.

For the signal readout, optical and electrochemical detection methods are mainly employed. The ability to perform medical diagnosis at the bedside or at the point-of-care is important in health care, especially in developing countries where access to centralized hospitals is limited and prohibitively expensive. To this end, point-of-care diagnostic bio-MEMS have been developed to
take saliva, blood, or urine samples and in an integrated approach perform sample preconditioning, sample fractionation, signal amplification, analyte detection, data analysis, and result display. In particular, blood is a very common biological sample because it cycles through the body every few minutes and its contents can indicate many aspects of health.

3.3.6. Sample conditioning

In blood analysis, white blood cells, platelets, bacteria, and plasma must be separated. Sieves, weirs, inertial confinement, and flow diversion devices are some approaches used in preparing blood plasma for cell-free analysis. Sieves can be microfabricated with high-aspect-ratio columns or posts, but are only suitable for low loading to avoid clogging with cells. Weirs are shallow mesa-like sections used to restrict flow to narrow slots between layers without posts. One advantage of using weirs is that the absence of posts allows more effective recycling of retenate for flow across the filter to wash off clogged cells. The H-filter is a microfluidic device with two inlets and two outlets that takes advantage of laminar flow and diffusion to separate components that diffuse across the interface between two inlet streams. By controlling the flow rate, diffusion distance, and residence time of the fluid in the filter, cells are excluded from the filtrate by virtue of their slower diffusion rate. The H-filter does not clog and can run indefinitely, but analytes are diluted by a factor of two. For cell analysis, cells can be studied intact or after lysis. A lytic buffer stream can be introduced alongside a stream containing cells and by diffusion induces lysis prior to further analysis. Cell analysis is typically done by flow cytometry and can be implemented into microfluidics with lower fluid velocities and lower throughput than their conventional macroscopic counterparts.

3.3.7. Sample fractionation

Microfluidic sample separation can be achieved by capillary electrophoresis or continuous-flow separation. In capillary electrophoresis, a long thin tube separates analytes by voltage as they migrate by electro-osmotic flow. For continuous-flow separation, the general idea is to apply a field at an angle to the flow direction to deflect the sample flow path toward different channels. Examples of continuous-flow separation techniques include continuous-flow electrophoresis, isoelectric focusing, continuous-flow magnetic separations, and molecular sieving.
CHAPTER 4: Bio-MEMS IN TISSUE ENGINEERING

4.1. Cell culture

The lung-on-a-chip device simulates the contraction of the diaphragm, which triggers the intrapleural pressure to decrease, leading to an expansion of alveoli.

*Cell culture in a special tissue culture dish*

*Epithelial cells in culture, stained for keratin (red) and DNA (green)*
Cell culture is the process by which cells are grown under controlled conditions, generally outside their natural environment. Cell culture conditions can vary for each cell type, but artificial environments consist of a suitable vessel with substrate or medium that supplies the essential nutrients (amino acids, carbohydrates, vitamins, minerals), growth factors, hormones, and gases (CO₂, O₂), and regulates the physio-chemical environment (pH buffer, osmotic pressure, temperature). Most cells require a surface or an artificial substrate (adherent or monolayer culture) whereas others can be grown free floating in culture medium (suspension culture). The lifespan of most cells is genetically determined, but some cell culturing cells have been “transformed” into immortal cells which will reproduce indefinitely if the optimal conditions are provided. In practice, the term "cell culture" now refers to the culturing of cells derived from multicellular eukaryotes, especially animal cells, in contrast with other types of culture that also grow cells, such as plant tissue culture, fungal culture, and microbiological culture (of microbes). The historical development and methods of cell culture are closely interrelated to those of tissue culture and organ culture. Viral culture is also related, with cells as hosts for the viruses. The laboratory technique of maintaining live cell lines (a population of cells descended from a single cell and containing the same genetic makeup) separated from their original tissue source became more robust in the middle 20th century.

4.1.1. History

The 19th-century English physiologist Sydney Ringer developed salt solutions containing the chlorides of sodium, potassium, calcium and magnesium suitable for maintaining the beating of an isolated animal heart outside the body. In 1885, Wilhelm Roux removed a portion of the medullary plate of an embryonic chicken and maintained it in a warm saline solution for several days, establishing the principle of tissue culture. Ross Granville Harrison, working at Johns Hopkins Medical School and then at Yale University, published results of his experiments from 1907 to 1910, establishing the methodology of tissue culture; Cell culture techniques were advanced significantly in the 1940s and 1950s to support research in virology. Growing viruses in cell cultures allowed preparation of purified viruses for the manufacture of vaccines. The injectable polio vaccine developed by Jonas Salk was one of the first products mass-produced using cell culture techniques. This vaccine was made possible by the cell culture research of John
Franklin Enders, Thomas Huckle Weller, and Frederick Chapman Robbins, who were awarded a Nobel Prize for their discovery of a method of growing the virus in monkey kidney cell cultures.

4.1.2. Concepts in mammalian cell culture

4.1.2.1. Isolation of cells

Cells can be isolated from tissues for *ex vivo* culture in several ways. Cells can be easily purified from blood; however, only the white cells are capable of growth in culture. Mononuclear cells can be released from soft tissues by enzymatic digestion with enzymes such as collagenase, trypsin, or pronase, which break down the extracellular matrix. Alternatively, pieces of tissue can be placed in growth media, and the cells that grow out are available for culture. This method is known as explant culture. Cells that are cultured directly from a subject are known as primary cells. With the exception of some derived from tumors, most primary cell cultures have limited lifespan. An established or immortalized cell line has acquired the ability to proliferate indefinitely either through random mutation or deliberate modification, such as artificial expression of the telomerase gene. Numerous cell lines are well established as representative of particular cell types.

4.1.2.2. Maintaining cells in culture

For the majority of isolated primary cells, they undergo the process of senescence and stop dividing after a certain number of population doublings while generally retaining their viability (described as the Hayflick limit). Cells are grown and maintained at an appropriate temperature and gas mixture (typically, 37 °C, 5% CO₂ for mammalian cells) in a cell incubator. Culture conditions vary widely for each cell type, and variation of conditions for a particular cell type can result in different phenotypes.

*A bottle of DMEM cell culture medium*
Aside from temperature and gas mixture, the most commonly varied factor in culture systems is the cell growth medium. Recipes for growth media can vary in pH, glucose concentration, growth factors, and the presence of other nutrients. The growth factors used to supplement media are often derived from the serum of animal blood, such as fetal bovine serum (FBS), bovine calf serum, equine serum, and porcine serum. One complication of these blood-derived ingredients is the potential for contamination of the culture with viruses or prions, particularly in medical biotechnology applications. Current practice is to minimize or eliminate the use of these ingredients wherever possible and use human platelet lysate (hPL). This eliminates the worry of cross-species contamination when using FBS with human cells. hPL has emerged as a safe and reliable alternative as a direct replacement for FBS or other animal serum. In addition, chemically defined media can be used to eliminate any serum trace (human or animal), but this cannot always be accomplished with different cell types. Alternative strategies involve sourcing the animal blood from countries with minimum BSE/TSE risk, such as The United States, Australia and New Zealand, and using purified nutrient concentrates derived from serum in place of whole animal serum for cell culture. Plating density (number of cells per volume of culture medium) plays a critical role for some cell types. For example, a lower plating density makes granulosa cells exhibit estrogen production, while a higher plating density makes them appear as progesterone-producing theca lutein cells.

Cells can be grown either in suspension or adherent cultures. Some cells naturally live in suspension, without being attached to a surface, such as cells that exist in the bloodstream. There are also cell lines that have been modified to be able to survive in suspension cultures so they can be grown to a higher density than adherent conditions would allow. Adherent cells require a surface, such as tissue culture plastic or microcarrier, which may be coated with extracellular matrix (such as collagen and laminin) components to increase adhesion properties and provide other signals needed for growth and differentiation. Most cells derived from solid tissues are adherent. Another type of adherent culture is organotypic culture, which involves growing cells in a three-dimensional (3-D) environment as opposed to two-dimensional culture dishes. This 3D culture system is biochemically and physiologically more similar to in vivo tissue, but is technically challenging to maintain because of many factors (e.g. diffusion).
4.1.2.3. Components of cell culture media

<table>
<thead>
<tr>
<th>Component</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon source (glucose/glutamine)</td>
<td>Source of energy</td>
</tr>
<tr>
<td>Amino acid</td>
<td>Building blocks of protein</td>
</tr>
<tr>
<td>Vitamins</td>
<td>Promote cell survival and growth</td>
</tr>
<tr>
<td>Balanced salt solution</td>
<td>An isotonic mixture of ions to maintain optimum osmotic pressure within the cells and provide essential metal ions to act as cofactors for enzymatic reactions, cell adhesion etc.</td>
</tr>
<tr>
<td>Phenol red dye</td>
<td>pH indicator. The color of phenol red changes from orange/red at pH 7-7.4 to yellow at acidic (lower) pH and purple at basic (higher) pH.</td>
</tr>
<tr>
<td>Bicarbonate/HEPES buffer</td>
<td>It is used to maintain a balanced pH in the media</td>
</tr>
</tbody>
</table>

i. Typical Growth conditions

<table>
<thead>
<tr>
<th>Parameter</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>37 °C</td>
</tr>
<tr>
<td>CO2</td>
<td>5%</td>
</tr>
<tr>
<td>Humidity</td>
<td>95%</td>
</tr>
</tbody>
</table>

4.1.2.4. Cell line cross-contamination

Cell line cross-contamination can be a problem for scientists working with cultured cells. Studies suggest anywhere from 15–20% of the time, cells used in experiments have been misidentified or
contaminated with another cell line. Problems with cell line cross-contamination have even been detected in lines from the NCI-60 panel, which are used routinely for drug-screening studies. Major cell line repositories, including the American Type Culture Collection (ATCC), the European Collection of Cell Cultures (ECACC) and the German Collection of Microorganisms and Cell Cultures (DSMZ), have received cell line submissions from researchers that were misidentified by them. Such contamination poses a problem for the quality of research produced using cell culture lines, and the major repositories are now authenticating all cell line submissions. ATCC uses short tandem repeat (STR) DNA fingerprinting to authenticate its cell lines. To address this problem of cell line cross-contamination, researchers are encouraged to authenticate their cell lines at an early passage to establish the identity of the cell line. Authentication should be repeated before freezing cell line stocks, every two months during active culturing and before any publication of research data generated using the cell lines. Many methods are used to identify cell lines, including isoenzyme analysis, human lymphocyte antigen (HLA) typing, chromosomal analysis, karyotyping, morphology, and STR analysis. One significant cell-line cross contaminant is the immortal HeLa cell line.

4.1.2.5. Other technical issues

As cells generally continue to divide in culture, they generally grow to fill the available area or volume.

This can generate several issues:

- Nutrient depletion in the growth media
- Changes in pH of the growth media
- Accumulation of apoptotic/necrotic (dead) cells
- Cell-to-cell contact can stimulate cell cycle arrest, causing cells to stop dividing, known as contact inhibition.
- Cell-to-cell contact can stimulate cellular differentiation.
- Genetic and epigenetic alterations, with a natural selection of the altered cells potentially leading to overgrowth of abnormal, culture-adapted cells with decreased differentiation and increased proliferative capacity.
4.1.2.6. Manipulation of cultured cells

Among the common manipulations carried out on culture cells are media changes, passaging cells, and transfecting cells. These are generally performed using tissue culture methods that rely on aseptic technique. Aseptic technique aims to avoid contamination with bacteria, yeast, or other cell lines. Manipulations are typically carried out in a biosafety hood or laminar flow cabinet to exclude contaminating micro-organisms. Antibiotics (e.g. penicillin and streptomycin) and antifungals (e.g. amphotericin B) can also be added to the growth media. As cells undergo metabolic processes, acid is produced and the pH decreases. Often, a pH indicator is added to the medium to measure nutrient depletion.

i. Media changes

In the case of adherent cultures, the media can be removed directly by aspiration, and then is replaced. Media changes in non-adherent cultures involve centrifuging the culture and resuspending the cells in fresh media.

ii. Passaging cells

Passaging (also known as subculture or splitting cells) involves transferring a small number of cells into a new vessel. Cells can be cultured for a longer time if they are split regularly, as it avoids the senescence associated with prolonged high cell density. Suspension cultures are easily passaged with a small amount of culture containing a few cells diluted in a larger volume of fresh media. For adherent cultures, cells first need to be detached; this is commonly done with a mixture of trypsin-EDTA; however, other enzyme mixes are now available for this purpose. A small number of detached cells can then be used to seed a new culture. Some cell cultures, such as RAW cells are mechanically scraped from the surface of their vessel with rubber scrapers.

iii. Transfection and transduction

Another common method for manipulating cells involves the introduction of foreign DNA by transfection. This is often performed to cause cells to express a gene of interest. More recently, the transfection of RNAi constructs have been realized as a convenient mechanism for
suppressing the expression of a particular gene/protein. DNA can also be inserted into cells using viruses, in methods referred to as transduction, infection or transformation. Viruses, as parasitic agents, are well suited to introducing DNA into cells, as this is a part of their normal course of reproduction.

4.1.2.7. Established human cell lines

Cultured HeLa cells have been stained with Hoechst turning their nuclei blue, and are one of the earliest human cell lines descended from Henrietta Lacks, who died of cervical cancer from which these cells originated.

Cell lines that originate with humans have been somewhat controversial in bioethics, as they may outlive their parent organism and later be used in the discovery of lucrative medical treatments. In the pioneering decision in this area, the Supreme Court of California held in Moore v. Regents of the University of California that human patients have no property rights in cell lines derived from organs removed with their consent. It is possible to fuse normal cells with an immortalised cell line. This method is used to produce monoclonal antibodies. In brief, lymphocytes isolated from the spleen (or possibly blood) of an immunised animal are combined with an immortal myeloma cell line (B cell lineage) to produce a hybridoma which has the antibody specificity of the primary lymphocyte and the immortality of the myeloma. Selective growth medium (HA or HAT) is used to select against unfused myeloma cells; primary lymphocytes die quickly in culture and only the fused cells survive. These are screened for production of the required antibody, generally in pools to start with and then after single cloning.
4.1.2.8. Cell strains

A cell strain is derived either from a primary culture or a cell line by the selection or cloning of cells having specific properties or characteristics which must be defined. Cell strains are cells that have been adapted to culture but, unlike cell lines, have a finite division potential. Non-immortalized cells stop dividing after 40 to 60 population doublings and, after this, they lose their ability to proliferate (a genetically determined event known as senescence).

4.1.3. Applications of cell culture

Mass culture of animal cell lines is fundamental to the manufacture of viral vaccines and other products of biotechnology. Culture of human stem cells is used to expand the number of cells and differentiate the cells into various somatic cell types for transplantation. Stem cell culture is also used to harvest the molecules and exosomes that the stem cells release for the purposes of therapeutic development. Biological products produced by recombinant DNA (rDNA) technology in animal cell cultures include enzymes, synthetic hormones, immunobiologicals (monoclonal antibodies, interleukins, lymphokines), and anticancer agents. Although many simpler proteins can be produced using rDNA in bacterial cultures, more complex proteins that are glycosylated (carbohydrate-modified) currently must be made in animal cells. An important example of such a complex protein is the hormone erythropoietin. The cost of growing mammalian cell cultures is high, so research is underway to produce such complex proteins in insect cells or in higher plants, use of single embryonic cell and somatic embryos as a source for direct gene transfer via particle bombardment, transit gene expression and confocal microscopy observation is one of its applications. It also offers to confirm single cell origin of somatic embryos and the asymmetry of the first cell division, which starts the process. Cell culture is also a key technique for cellular agriculture, which aims to provide both new products and new ways of producing existing agricultural products like milk, (cultured) meat, fragrances, and rhino horn from cells and microorganisms. It is therefore considered one means of achieving animal-free agriculture. It is also a central tool for teaching cell biology.
4.1.3.1. Cell culture in two dimensions

Research in tissue engineering, stem cells and molecular biology primarily involves cultures of cells on flat plastic dishes. This technique is known as two-dimensional (2D) cell culture, and was first developed by Wilhelm Roux who, in 1885, removed a portion of the medullary plate of an embryonic chicken and maintained it in warm saline for several days on a flat glass plate. From the advance of polymer technology arose today’s standard plastic dish for 2D cell culture, commonly known as the Petri dish. Julius Richard Petri, a German bacteriologist, is generally credited with this invention while working as an assistant to Robert Koch. Various researchers today also utilize culturing laboratory flasks, conicals, and even disposable bags like those used in single-use bioreactors. Aside from Petri dishes, scientists have long been growing cells within biologically derived matrices such as collagen or fibrin, and more recently, on synthetic hydrogels such as polyacrylamide or PEG. They do this in order to elicit phenotypes that are not expressed on conventionally rigid substrates. There is growing interest in controlling matrix stiffness, a concept that has led to discoveries in fields such as:

- Stem cell self-renewal
- Lineage specification
- Cancer cell phenotype
- Fibrosis
- Hepatocyte function
- Mechanosensing

4.1.3.2. Cell culture in three dimensions

Cell culture in three dimensions has been touted as "Biology's New Dimension". At present, the practice of cell culture remains based on varying combinations of single or multiple cell structures in 2D. Currently, there is an increase in use of 3D cell cultures in research areas including drug discovery, cancer biology, regenerative medicine and basic life science research. 3D cell cultures can be grown using a scaffold or matrix, or in a scaffold-free manner. Scaffold based cultures utilize an acellular 3D matrix or a liquid matrix. Scaffold-free methods are normally generated in suspensions. There are a variety of platforms used to facilitate the growth
of three-dimensional cellular structures including scaffold systems such as hydrogel matrices and solid scaffolds, and scaffold-free systems such as low-adhesion plates, nanoparticle facilitated magnetic levitation, and hanging drop plates.

i. 3D cell culture in scaffolds

Eric Simon, in a 1988 NIH SBIR grant report, showed that electrospinning could be used to produce nano- and submicron-scale polystyrene and polycarbonate fibrous scaffolds specifically intended for use as in vitro cell substrates. This early use of electrospun fibrous lattices for cell culture and tissue engineering showed that various cell types including Human Foreskin Fibroblasts (HFF), transformed Human Carcinoma (HEp-2), and Mink Lung Epithelium (MLE) would adhere to and proliferate upon polycarbonate fibers. It was noted that, as opposed to the flattened morphology typically seen in 2D culture, cells grown on the electrospun fibers exhibited a more histotypic rounded 3-dimensional morphology generally observed in vivo.

ii. 3D cell culture in hydrogels

As the natural extracellular matrix (ECM) is important in the survival, proliferation, differentiation and migration of cells, different hydrogel culture matrices mimicking natural ECM structure are seen as potential approaches to in vivo like cell culturing. Hydrogels are composed of interconnected pores with high water retention, which enables efficient transport of substances such as nutrients and gases. Several different types of hydrogels from natural and synthetic materials are available for 3D cell culture, including animal ECM extract hydrogels, protein hydrogels, peptide hydrogels, polymer hydrogels, and wood-based nanocellulose hydrogel.

iii. 3D Cell Culturing by Magnetic Levitation

The 3D Cell Culturing by Magnetic Levitation method (MLM) is the application of growing 3D tissue by inducing cells treated with magnetic nanoparticle assemblies in spatially varying magnetic fields using neodymium magnetic drivers and promoting cell to cell interactions by levitating the cells up to the air/liquid interface of a standard petri dish. The magnetic nanoparticle assemblies consist of magnetic iron oxide nanoparticles, gold nanoparticles, and the
polymer polylysine. 3D cell culturing is scalable, with the capability for culturing 500 cells to millions of cells or from single dish to high-throughput low volume systems.

4.1.3.3. Tissue culture and engineering

Cell culture is a fundamental component of tissue culture and tissue engineering, as it establishes the basics of growing and maintaining cells in vitro. The major application of human cell culture is in stem cell industry, where mesenchymal stem cells can be cultured and cryopreserved for future use. Tissue engineering potentially offers dramatic improvements in low cost medical care for hundreds of thousands of patients annually.

4.1.3.4. Vaccines

Vaccines for polio, measles, mumps, rubella, and chickenpox are currently made in cell cultures. Due to the H5N1 pandemic threat, research into using cell culture for influenza vaccines is being funded by the United States government. Novel ideas in the field include recombinant DNA-based vaccines, such as one made using human adenovirus (a common cold virus) as a vector, and novel adjuvants.

4.1.4. Culture of non-mammalian cells

Besides the culture of well-established immortalised cell lines, cells from primary explants of a plethora of organisms can be cultured for a limited period of time before senescence occurs (see Hayflick's limit). Cultured primary cells have been extensively used in research, as is the case of fish keratocytes in cell migration studies.

4.1.4.1. Plant cell culture methods

Plant cell cultures are typically grown as cell suspension cultures in a liquid medium or as callus cultures on a solid medium. The culturing of undifferentiated plant cells and calli requires the proper balance of the plant growth hormones auxin and cytokinin.
4.1.4.2. Insect cell culture

Cells derived from Drosophila melanogaster (most prominently, Schneider 2 cells) can be used for experiments which may be hard to do on live flies or larvae, such as biochemical studies or studies using siRNA. Cell lines derived from the army worm Spodoptera frugiperda, including Sf9 and Sf21, and from the cabbage looper Trichoplusia ni, High Five cells, are commonly used for expression of recombinant proteins using baculovirus.

4.1.4.3. Bacterial and yeast culture methods

For bacteria and yeasts, small quantities of cells are usually grown on a solid support that contains nutrients embedded in it, usually a gel such as agar, while large-scale cultures are grown with the cells suspended in a nutrient broth.

4.1.4.4. Viral culture methods

The culture of viruses requires the culture of cells of mammalian, plant, fungal or bacterial origin as hosts for the growth and replication of the virus. Whole wild type viruses, recombinant viruses or viral products may be generated in cell types other than their natural hosts under the right conditions. Depending on the species of the virus, infection and viral replication may result in host cell lysis and formation of a viral plaque.

4.1.5. Common cell lines

Human cell lines

- DU145 (prostate cancer)
- H295R (adrenocortical cancer)
- HeLa (cervical cancer)
- KBM-7 (chronic myelogenous leukemia)
- LNCaP (prostate cancer)
- MCF-7 (breast cancer)
- MDA-MB-468 (breast cancer)
- PC3 (prostate cancer)
- SaOS-2 (bone cancer)
- SH-SY5Y (neuroblastoma, cloned from a myeloma)
- T47D (breast cancer)
- THP-1 (acute myeloid leukemia)
- U87 (glioblastoma)
- National Cancer Institute's 60 cancer cell line panel (NCI60)

Primate cell lines

- Vero (African green monkey *Chlorocebus* kidney epithelial cell line)

Mouse cell lines

- MC3T3 (embryonic calvarium)

Rat tumor cell lines

- GH3 (pituitary tumor)
- PC12 (pheochromocytoma)

Plant cell lines

- Tobacco BY-2 cells (kept as cell suspension culture, they are model system of plant cell)

Other species cell lines

- Dog MDCK kidney epithelial
- Xenopus A6 kidney epithelial
- Zebrafish AB9

### 4.1.6. List of cell lines

This list is incomplete; you can help by expanding it.
<table>
<thead>
<tr>
<th>Cell line</th>
<th>Meaning</th>
<th>Organism</th>
<th>Origin tissue</th>
<th>Morphology</th>
<th>Links</th>
</tr>
</thead>
<tbody>
<tr>
<td>3T3-L1</td>
<td>&quot;3-day transfer, inoculum 3 x 10^5 cells&quot;</td>
<td>Mouse</td>
<td>Embryo</td>
<td>Fibroblast</td>
<td>ECACC Cellosaurus</td>
</tr>
<tr>
<td>4T1</td>
<td></td>
<td>Mouse</td>
<td>Mammary gland</td>
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<tr>
<td>9L</td>
<td></td>
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<td>Brain</td>
<td>Glioblastoma</td>
<td>ECACC Cellosaurus</td>
</tr>
<tr>
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<td>Glioblastoma</td>
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<td>B lymphoma</td>
<td>B lymphocyte</td>
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<td>ECACC Cellosaurus</td>
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<td>Ovary</td>
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4.1.7. **Cell culture assay**

In biomaterials testing, a cell culture assay is any method which is used to assess the cytotoxicity of a material. This refers to the *in vitro* assessment of material to determine whether it releases toxic chemicals in sufficient quantities to kill cells either directly or indirectly through the inhibition of cell metabolic pathways. Cell culture evaluations are the precursor to whole animal studies and are a way to determine if significant cytotoxicity exists for the given material. Cell culture assays are standardized by ASTM, ISO, and BSI (British Standards Institution.)

4.1.7.1. **Methods**

i. **Direct contact method**

1. A near confluent layer of fibroblasts are prepared in a culture plate
2. Old cell culture media ([agar] generally) is removed
3. Fresh media is added
4. Material being tested is placed onto the cultures, which are incubated for 24 hours at 37 degrees Celsius
5. The material is removed
6. The culture media is removed
7. The remaining cells are fixed and stained, dead cells are lost during fixation and only the live cells are stained
8. The toxicity of the material is indicated by the absence of stained cells around the material.

**ii. Agar diffusion method**

1. A near confluent layer of fibroblasts are prepared in a culture plate
2. Old cell culture media is removed
3. The cells are covered with a solution of 2% agar, which often contains red vital stain
4. When the agar solidifies the cells will have dispersed throughout its volume
5. The material is then placed on the surface of the agar and incubated for 24 hours at 37 degrees Celsius
6. Live cells take up the vital stain and retain it, dead cells do not
7. The toxicity of the material is evaluated by the loss of vital stain under and around the material
8. Surface microscopy is also needed to evaluate the material-cell interface

**iii. Elution method**

1. A near confluent layer of fibroblasts are prepared in a culture plate.
2. An extract of the material which is being tested is prepared using physiological saline or serum free media (the latter is generally preferred).
3. Extraction conditions are used which are appropriate for the type of exposure which the cells would receive in the *in vivo* environment if the material were to be implanted.
4. The extract is placed on the cells and incubated for 48 hours at 37 degrees Celsius.
5. After 48 hours the toxicity is evaluated using either a histo-chemical or vital stain.
Each method has its own advantages and disadvantages, and some are more suitable for certain applications than others. For example the direct contact method offers conditions which are most similar to the physiological environment but the cells are susceptible to trauma if the material moves. The agar diffusion method is good for materials with high densities and offers an even concentration gradient for potential toxicants, but there is a serious risk of the cells going into thermal shock when they are overlaid with agar. The elution method is best for applications which might require extra incubation time, but additional time and steps are required for preparing such a test. *In vitro* biomaterials testing yields fundamental information about the behavior of materials in contact with living cells, but cannot qualify or even accurately predict the performance of a material *in vivo*.

Conventional cell culture technology is unable to efficiently allow combinatorial testing of drug candidates, growth factors, neuropeptides, genes, and retroviruses in cell culture medium. Due to the need for cells to be fed periodically with fresh medium and passaged, even testing a few conditions requires a large number of cells and supplies, expensive and bulky incubators, large fluid volumes (~0.1 – 2 mL per sample), and tedious human labour. The requirement of human labour also limits the number and length between time points for experiments. Microfluidic cell cultures are potentially a vast improvement because they can be automated, as well as yield lower overall cost, higher throughput, and more quantitative descriptions of single-cell behaviour variability. By including gas exchange and temperature control systems on chip, microfluidic cell culturing can eliminate the need for incubators and tissue culture hoods. However, this type of continuous microfluidic cell culture operation presents its own unique challenges as well. Flow control is important when seeding cells into microchannels because flow needs to be stopped after the initial injection of cell suspension for cells to attach or become trapped in microwells, dielectrophoretic traps, micromagnetic traps, or hydrodynamic traps.

Subsequently, flow needs to be resumed in a way that does not produce large forces that shear the cells off the substrate. Dispensing fluids by manual or robotic pipetting can be replaced with micropumps and microvalves, where fluid metering is straightforward to determine as opposed to continuous flow systems by micromixers. A fully automated microfluidic cell culture system has been developed to study osteogenic differentiation of human embryonic stem cells. A handheld microfluidic cell culture incubator capable of heating and pumping cell culture
solutions has also been developed. Due to the volume reduction in microfluidic cultures, the collected concentrations are higher for better signal-to-noise ratio measurements, but collection and detection is correspondingly more difficult. *In situ* microscopy assays with microfluidic cell cultures may help in this regard, but have inherently lower throughput due to the microscope probe having only a small field of view.

Micropatterned co-cultures have also contributed to bio-MEMS for tissue engineering to recapitulate *in vivo* conditions and 3D natural structure. Specifically, hepatocytes have been patterned to co-culture at specific cell densities with fibroblasts to maintain liver-specific functions such as albumin secretion, urea synthesis, and p450 detoxification. Similarly, integrating microfluidics with micropatterned co-cultures has enabled modelling of organs where multiple vascularized tissues interface, such as the blood–brain barrier and the lungs. Organ-level lung functions have been reconstituted on lung-on-a-chip devices where a porous membrane and the seeded epithelial cell layer are cyclically stretched by applied vacuum on adjacent microchannels to mimic inhalation.

### 4.2. Stem-cell engineering

An integrated microfluidic device with a concentration gradient generator and individual cell chambers for studying dose-dependent effects of differentiation inducing factors
The goal of stem cell engineering is to be able to control the differentiation and self-renewal of pluripotency stem cells for cell therapy. Differentiation in stem cells is dependent on many factors, including soluble and biochemical factors, fluid shear stress, cell-ECM interactions, cell-cell interactions, as well as embryoid body formation and organization. Bio-MEMS have been used to research how to optimize the culture and growth conditions of stem cells by controlling these factors. Assaying stem cells and their differentiated progeny is done with microarrays for studying how transcription factors and miRNAs determine cell fate, how epigenetic modifications between stem cells and their daughter cells affect phenotypes, as well as measuring and sorting stem cells by their protein expression.

4.2.1. **Biochemical factors**

Microfluidics can leverage its microscopic volume and laminar flow characteristics for spatiotemporal control of biochemical factors delivered to stem cells. Microfluidic gradient generators have been used to study dose-response relationships. Oxygen is an important biochemical factor to consider in differentiation via hypoxia-induced transcription factors (HIFs) and related signaling pathways, most notably in the development of blood, vasculature, placental, and bone tissues. Conventional methods of studying oxygen effects relied on setting the entire incubator at a particular oxygen concentration, which limited analysis to pair-wise comparisons between normoxic and hypoxic conditions instead of the desired concentration-dependent characterization. Developed solutions include the use of continuous axial oxygen gradients and arrays of microfluidic cell culture chambers separated by thin PDMS membranes to gas-filled microchannels.

4.2.2. **Fluid shear stress**

Fluid shear stress is relevant in the stem cell differentiation of cardiovascular lineages as well as late embryogenesis and organogenesis such as left-right asymmetry during development. Macro-scale studies do not allow quantitative analysis of shear stress to differentiation because they are performed using parallel-plate flow chambers or rotating cone apparatuses in on-off scenarios only. Poiseuille flow in microfluidics allows shear stresses to be varied systematically using
channel geometry and flow rate via micropumps, as demonstrated by using arrays of perfusion chambers for mesenchymal stem cells and fibroblast cell adhesion studies.

### 4.2.3. Cell–ECM interactions

Cell-ECM interactions induce changes in differentiation and self-renewal by the stiffness of the substrate via mechanotransduction, and different integrins interacting with ECM molecules. Micropatterning of ECM proteins by micro-contact printing (μCP), inkjet printing, and mask spraying have been used in stem cell-ECM interaction studies. It has been found by using micro-contact printing to control cell attachment area that that switch in osteogenic / adipogenic lineage in human mesenchymal stem cells can be cell shape dependent. Microfabrication of microposts and measurement of their deflection can determine traction forces exerted on cells. Photolithography can also be used to cross-link cell-seeded photo-polymerizable ECM for three-dimensional studies. Using ECM microarrays to optimize combinatorial effects of collagen, laminin, and fibronectin on stem cells is more advantageous than conventional well plates due to its higher throughput and lower requirement of expensive reagents.

### 4.2.4. Cell–cell interactions

Cell fate is regulated by both interactions between stem cells and interactions between stem cells and membrane proteins. Manipulating cell seeding density is a common biological technique in controlling cell–cell interactions, but controlling local density is difficult and it is often difficult to decouple effects between soluble signals in the medium and physical cell–cell interactions. Micropatterning of cell adhesion proteins can be used in defining the spatial positions of different cells on a substrate to study human ESC proliferation. Seeding stem cells into PDMS microwells and flipping them onto a substrate or another cell layer is a method of achieving precise spatial control. Gap junction communications has also been studied using microfluidics whereby negative pressure generated by fluid flow in side channels flanking a central channel traps pairs of cells that are in direct contact or separated by a small gap. However, in general, the non-zero motility and short cell cycle time of stem cells often disrupt the spatial organization imposed by these microtechnologies.
Murine embryoid bodies in suspension culture after 24 hours of formation from embryonic stem cells

4.2.5. Embryoid body formation and organization

Embryoid bodies are a common in vitro pluripotency test for stem cells and their size needs to be controlled to induce directed differentiation to specific lineages. High throughput formation of uniform sized embryoid bodies with microwells and microfluidics allows easy retrieval and more importantly, scale up for clinical contexts. Actively controlling embryoid body cell organization and architecture can also direct stem cell differentiation using microfluidic gradients of endoderm-, mesoderm- and ectoderm-inducing factors, as well as self-renewal factors.

4.3. Assisted reproductive technologies

Assisted reproductive technologies help to treat infertility and genetically improve livestock. However, the efficiency of these technologies in cryopreservation and the in vitro production of mammalian embryos are low. Microfluidics has been applied in these technologies to better mimic the in vivo microenvironment with patterned topographic and biochemical surfaces for controlled spatiotemporal cell adhesion, as well as minimization of dead volumes. Micropumps and microvalves can automate tedious fluid-dispensing procedures and various sensors can be integrated for real-time quality control. Bio-MEMS devices have been developed to evaluate sperm motility, perform sperm selection, as well as prevent polyspermy in in-vitro fertilization.
CHAPTER 5: Bio-MEMS IN MEDICAL IMPLANTS AND SURGERY

5.1. Implantable microelectrodes

The goal of implantable microelectrodes is to interface with the body’s nervous system for recording and sending bioelectrical signals to study disease, improve prostheses, and monitor clinical parameters. Microfabrication has led to the development of Michigan probes and the Utah electrode array, which have increased electrodes per unit volume, while addressing problems of thick substrates causing damage during implantation and triggering foreign-body reaction and electrode encapsulation via silicon and metals in the electrodes. Michigan probes have been used in large-scale recordings and network analysis of neuronal assemblies and the Utah electrode array has been used as a brain–computer interface for the paralyzed. Extracellular microelectrodes have been patterned onto an inflatable helix-shaped plastic in cochlear implants to improve deeper insertion and better electrode-tissue contact for transduction of high-fidelity sounds. Integrating microelectronics onto thin, flexible substrates has led to the development of a cardiac patch that adheres to the curvilinear surface of the heart by surface tension alone for measuring cardiac electrophysiology, and electronic tattoos for measuring skin temperature and bioelectricity.

A cardiac balloon catheter with temperature sensors, electrocardiography sensors, and LEDs is surgical bio-MEMS.
5.2. Microtools for surgery

Bio-MEMS for surgical applications can improve existing functionality, add new capabilities for surgeons to develop new techniques and procedures, and improve surgical outcomes by lowering risk and providing real-time feedback during the operation. Micromachined surgical tools such as tiny forceps, microneedle arrays and tissue debriders have been made possible by metal and ceramic layer-by-layer microfabrication techniques for minimally invasive surgery and robotic surgery. Incorporation of sensors onto surgical tools also allows tactile feedback for the surgeon, identification of tissue type via strain and density during cutting operations, and diagnostic catheterization to measure blood flows, pressures, temperatures, oxygen content, and chemical concentrations.

5.3. Drug delivery

Drug delivery refers to approaches, formulations, technologies, and systems for transporting a pharmaceutical compound in the body as needed to safely achieve its desired therapeutic effect. It may involve scientific site-targeting within the body, or it might involve facilitating systemic pharmacokinetics; in any case, it is typically concerned with both quantity and duration of drug presence. Drug delivery is often approached via a drug's chemical formulation, but it may also involve medical devices or drug-device combination products. Drug delivery is a concept heavily integrated with dosage form and route of administration, the latter sometimes even being considered part of the definition. Drug delivery technologies modify drug release profile, absorption, distribution and elimination for the benefit of improving product efficacy and safety, as well as patient convenience and compliance. Drug release is from: diffusion, degradation, swelling, and affinity-based mechanisms. Most common routes of administration include the preferred non-invasive peroral (through the mouth), topical (skin), transmucosal (nasal, buccal/sublingual, vaginal, ocular and rectal) and inhalation routes. Many medications such as peptide and protein, antibody, vaccine and gene based drugs, in general may not be delivered using these routes because they might be susceptible to enzymatic degradation or cannot be absorbed into the systemic circulation efficiently due to molecular size and charge issues to be therapeutically effective. For this reason many protein and peptide drugs have to be delivered by
injection or a nanoneedle array. For example, many immunizations are based on the delivery of protein drugs and are often done by injection.

Current efforts in the area of drug delivery include the development of targeted delivery in which the drug is only active in the target area of the body (for example, in cancerous tissues), sustained release formulations in which the drug is released over a period of time in a controlled manner from a formulation, and methods to increase survival of peroral agents which must pass through the stomach's acidic environment. In order to achieve efficient targeted delivery, the designed system must avoid the host's defense mechanisms and circulate to its intended site of action. Types of sustained release formulations include liposomes, drug loaded biodegradable microspheres and drug polymer conjugates. Survival of agents as they pass through the stomach typically is an issue for agents who cannot be encased in a solid tablet; one research area has been around the utilization of lipid isolates from the acid-resistant archaea Sulfolobus islandicus, which confers on the order of 10% survival of liposome-encapsulated agents.

Transdermal microneedles patch is less invasive compared to conventional drug delivery by hypodermic needle.

Microneedles, formulation systems, and implantable systems are bio-MEMS applicable to drug delivery. Microneedles of approximately 100μm can penetrate the skin barrier and deliver drugs to the underlying cells and interstitial fluid with reduced tissue damage, reduced pain, and no bleeding. Microneedles can also be integrated with microfluidics for automated drug loading or multiplexing. From the user standpoint, microneedles can be incorporated into a patch format for self-administration, and do not constitute a sharp waste biohazard (if the material is polymeric). Drug delivery by microneedles include coating the surface with therapeutic agents, loading drugs into porous or hollow microneedles, or fabricating the microneedles with drug and coating
matrix for maximum drug loading. Microneedles for interstitial fluid extraction, blood extraction, and gene delivery are also being developed. The efficiency of microneedle drug delivery remains a challenge because it is difficult to ascertain if the microneedles effectively penetrated the skin. Some drugs, such as diazepam, are poorly soluble and need to be aerosolized immediately prior to intranasal administration. Bio-MEMS technology using piezoelectric transducers to liquid reservoirs can be used in these circumstances to generate narrow size distribution of aerosols for better drug delivery. Implantable drug delivery systems have also been developed to administer therapeutic agents that have poor bioavailability or require localized release and exposure at a target site. Examples include a PDMS microfluidic device implanted under the conjunctiva for drug delivery to the eye to treat ocular diseases and microchips with gold-capped drug reservoirs for osteoporosis. In implantable bio-MEMS for drug delivery, it is important to consider device rupture and dose dumping, fibrous encapsulation of the device, and device explantation. Most drugs also need to be delivered in relatively large quantities (milliliters or even greater), which makes implantable bio-MEMS drug delivery challenging due to their limited drug-holding capacity.
REFERENCES


Hochberg, Leigh R.; Serruya, Mijail D.; Friehs, Gerhard M.; Mukand, Jon A.; Saleh, Maryam; Caplan, Abraham H.; Branner, Almut; Chen, David; Penn, Richard D.; Donoghue, John P.


Rubinsky, Boris; Aki, Atsushi; Nair, Baiju G.; Morimoto, Hisao; Kumar, D. Sakthi; Maekawa, Toru (2010). "Label-Free Determination of the Number of Biomolecules Attached to Cells by Measurement of the Cell's Electrophoretic Mobility in a Microchannel". PLoS ONE. 5 (12): e15641.


Torisawa, Yu-suke; Chueh, Bor-han; Huh, Dongeun; Ramamurthy, Poornapriya; Roth, Therese M.; Barald, Kate F.; Takayama, Shuichi (2007). "Efficient formation of uniform-sized embryoid bodies using a compartmentalized microchannel device". Lab on a Chip. 7 (6): 770–6.


Wanunu, Meni; Cao, Qingqing; Mahalanabis, Madhumita; Chang, Jessie; Carey, Brendan; Hsieh, Christopher; Stanley, Ahjegannie; Odell, Christine A.; Mitchell, Patricia; Feldman, James; Pollock, Nira R.; Klapperich, Catherine M. (2012). "Microfluidic Chip for Molecular Amplification of Influenza A RNA in Human Respiratory Specimens". PLoS ONE. 7 (3): e33176.


Yang, Yanmin; Tian, Xiliang; Wang, Shouyu; Zhang, Zhen; Lv, Decheng (2012). "Rat Bone Marrow-Derived Schwann-Like Cells Differentiated by the Optimal Inducers Combination on Microfluidic Chip and Their Functional Performance". PLoS ONE. 7 (8): e42804.