J. ISSS Vol. 3 No. 2, pp. 39-59, Sept. 2014. REVIEW ARTICLE

A Technology Overview and Applications of Bio-MEMS

Nidhi Maheshwari[+], **Gaurav Chatterjee**[+], **V. Ramgopal Rao**.
Department of Electrical Engineering,
Indian Institute of Technology Bombay,
Mumbai, India-400076.
Corresponding Author: rrao@ee.iitb.ac.in

[+] Both the authors have contributed equally.

Keywords:

Bio-MEMS, immobilization, cantilever, micro-fabrication, biosensor.

Abstract

Miniaturization of conventional technologies has long been understood to have many benefits, like: lower cost of production, lower form factor leading to portable applications, and lower power consumption. Micro/Nano fabrication has seen tremendous research and commercial activity in the past few decades buoyed by the silicon revolution. As an offset of the same fabrication platform, the Micro-electro-mechanical-systems (MEMS) technology was conceived to fabricate complex mechanical structures on a micro level. MEMS technology has generated considerable research interest recently, and has even led to some commercially successful applications. Almost every smart phone is now equipped with a MEMS accelerometer-gyroscope system. MEMS technology is now being used for realizing devices having biomedical applications. Such devices can be placed under a subset of MEMS called the Bio-MEMS (Biological MEMS). In this paper, a brief introduction to the Bio-MEMS technology and the current state of art applications is discussed.

1. Introduction

Generally, the Bio-MEMS can be defined as any system or device, which is fabricated using the micro-nano fabrication technology, and used for biomedical applications such as diagnostics, therapeutics, drug delivery or real time monitoring Bashir (2004). Any Bio-MEMS device can be broken down to two primary aspects, the sensor/actuator and the system (Figure 1).

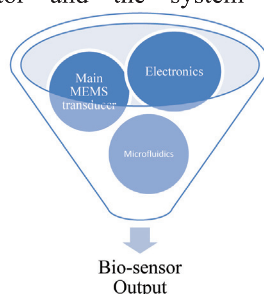

Figure 1: Basic system architecture of a biosensor.

The interdisciplinary nature of the Bio-MEMS research is highlighted in Figure 2. This highlights the overlapping of many different scientific disciplines, and the need for a healthy collaborative effort.

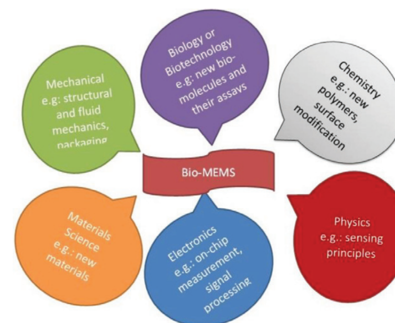

Figure 2: Interdisciplinary approach towards the Bio-MEMS realization.

The population explosion in the past three decades, especially in the developing countries has put a severe strain on the medical infrastructure of individual countries. Under such circumstances, a point of care rapid diagnostic solution is highly sought after. But the reduction in form factor of the sensor must not compromise on the sensitivity and accuracy of the system. Recent advances in MEMS technology have given researchers the ideal platform to conceptualise and develop miniaturised sensors, which are equally sensitive and accurate when compared to a traditional lab based diagnostic approach. In addition to the portability advantage, another direct outcome of MEMS is the ability to batch fabricate, driving the costs of these sensors to very low levels. These sensors, when coupled with adequate electronics, provide a very affordable and an accurate diagnostic option, especially to countries lacking an adequate medical facility penetration.

However, to research and develop a Bio-MEMS device, a coherent inter-disciplinary approach needs to be adopted. The chemistry and/or biotechnology experts need to develop a highly specific bio-capture strategy. This often depends on the bio-marker/ protein structure. Then the engineering experts need to devise a strategy for an efficient miniaturization of the transduction mechanism. The entire process flow also depends on the end use of the sensor owing to the fact that the certification procedures of bio-sensors are highly critical, intensive and elaborate. The process gets more complicated for invasive sensors. The materials chosen for invasive sensors need to be receptive to favourable biochemistry and at the same time must be bio-compatible. In addition to the biocompatibility challenge, they need to be MRI compatible (with the exception of few critical technologies, like the heart pacemaker). Such stringent certification laws pose a considerable challenge to the developers. The certification process also involves a lengthy and an intensive testing process. The entire procedure from proof of concept to a commercial product usually takes more than a decade.

The objective of this review is to give an introduction to some of the bio and electrical engineering solutions currently employed by researchers; to develop a bio-sensing device. An overview of the materials and MEMS fabrication technology is provided. Various sensing and bio-capture methods are discussed. Some microfluidics solutions are also discussed. However, the Bio-MEMS sensors have recently broadened their applications from only sensing to new innovative areas like minimally invasive surgery (Abushagur et al., 2014), therapeutics (Desai et al., 2007) and drug delivery (Staples et al., 2006, Shawgo et al., 2002, Nuxoll, 2013). These new applications are not discussed in this paper. However, readers are encouraged to refer to the associated references. Also, the certification processes involved, which vary in different countries, are outside the scope of this paper.

2. Materials and Fabrication Technology

MEMS/Bio-MEMS devices can be realized from a variety of materials, which can be grouped as : a) inorganic materials like silicon and glass, which have traditionally been used in the microelectronics fabrication, b) organic materials which include polymers like SU-8, PMMA, PDMS etc. and c) biomaterials like DNA, RNA and proteins which are utilized as biomimetic materials.

2.1 Materials

2.1.1 Silicon and glass

Inorganic materials like silicon and glass are traditionally widely used materials, due to the existing mature micromachining technology know-how. These materials also have good mechanical properties, which have been explored for the fabrication of devices like cantilevers, accelerometers, beams etc. Glass and silicon MEMS devices can be easily integrated with electronics on the same chip using the fabrication technology from IC industry. Additionally, glass is optically transparent, allowing: sensitive optical detection, feasibility of forming hermetically sealed devices due to a strong covalent bond formation, and a uniform and desirable surface characteristic for fluidic devices. However, glass processing is expensive, time consuming and complex. Owing to the amorphous nature of glass, wet etching forms isotropic structures and DRIE is very slow and limited in depth. Both glass and silicon are fragile materials; thereby, limiting their large scale use as field devices and are; also, expensive for fabrication so feasibility of disposable device formation cannot be fulfilled.

Newer materials, which can counter the limitations posed by silicon and glass, are highly desirable for the Bio-MEMS devices. Polymers are inexpensive, leading to the feasibility of disposable devices fabrication. Polymers can exist in a variety of forms, like: glassy state, hard, or soft/elastic state, which allows the formation of a variety of devices with arbitrary designs and 3-D structures, a property not present in the conventional silicon or glass substrates. Polymer based devices are less fragile and bio-compatible, which is an important criterion in case of prosthetics or in *vivo* diagnostics or in *vivo* drug delivery systems.

Another important advantage of using polymeric substrates is that their surface can be tailored as per the device/measurement needs, or choice of polymer can be made from a plethora of polymers. Apart from these properties, polymers also have a good chemical stability, ease of fabrication, optical transparency, high electrical insulation and good thermal properties, which make them ideal for realizing the Bio-MEMS devices. They also enjoy the advantage of easy machining by non-photolithographic methods, leading to a decrease in cost and complexity of processes. The use of these materials is expanding at a steady pace in commercial devices fabrication, like: DNA or protein microarrays, or electrophoretic chips. Among, a variety of polymers, SU-8, PDMS, and PMMA have garnered a lot of attention for the Bio-MEMS devices.

2.1.2 SU-8

SU-8 is an epoxy based photo-patternable negative resist, which has found a widespread use in MEMS/ Bio-MEMS device fabrication due to its compatibility with conventional methods of fabrication and its ability to form mechanically stable high aspect ratio structures. There are a number of advantages of using SU-8 for MEMS device fabrication. SU-8 is bio-compatible, transparent to visible light, mechanically stable and shows good chemical resistance to most of the solvents once cross-linked. It has been widely used for the fabrication of lab-on-chip devices (Svasek et al., 2004, Pang et al., 2008, Bilenberg et al., 2004, Ruano-Lopez et al., 2006, Arscott, 2014), probes for scanning probe microscopy (Marie et al., 2006), optical waveguides (Prabhakar and Mukherji, 2010), microfluidic structures (Aguirregabiria et al., 2004, Yoon et al., 2008, Mali et al., 2006), nanomechanical sensors (Calleja et al., 2005, Joshi et al., 2010, Seena et al., 2009a, Thaysen et al., 2001), micro-electrophoresis chips, DNA and protein arrays (Wang et al., 2007) etc. In spite of a number of attractive features, SU-8 has some limitations in terms of self-fluorescence, which leads to background noise in case of optical measurements like fluorescence based assays. Formation of closed micro-channels with patterned structures like electrodes on either or both the sides of the channel requires bonding of two cross-linked SU-8 structures, which has very less reflow characteristic leading to an average bond quality. The surface hydrophobicity of SU-8 leads to poor wetting characteristic, leading to difficulty in filling aqueous fluids in microchannels and also leads to physical adsorption of bio-molecules like proteins. Due to hydrophobicity, cells are unable to attach and grow on the surface. Therefore, for using SU-8 as a structural material for bio-applications like micro-channels or cell sorter, the surface chemistry has to be modified.

2.1.3 Polydimethylsiloxane (PDMS)

PDMS belongs to the class of silicones; materials which have silicon and oxygen forming the major bulk of the material. It is a viscoelastic polymer [elastomer, with a molecular formula- $(C_2H_6OSi)_n$]. It is a low cost material and is stable against humidity variations. PDMS has attracted a lot of attention as a polymeric material for fabricating microfluidic devices in biological applications for a number of reasons: reproducible features on the micrometer scale can be produced with a high fidelity by cheaper fabrication routes, optical transparency down to 280nm, low temperature polymerization, bio-compatible nature, reversible deformation and self-sealing capability. It has a low interfacial energy, which prevents it from any unwanted reaction with the solution or other polymers. The surface properties of PDMS can be readily tailored by well proven surface modification protocols. In addition, their permeability to oxygen and carbon dioxide make this material well suited for cell growth systems.

PDMS in its native form is not well suited for micro-fluidic applications involving aqueous fluids: hydrophobic surface of the material results in PDMS being difficult to wet. Its low surface energy; also leads to adsorption of bio-molecules and other hydrophobic compounds, which can, in turn, lower the sensitivity of the device. The auto-fluorescence nature of PDMS limits its usage in the optical measurement set-ups. It also has a poor chemical stability, especially in presence of organic solvents, the bulk polymer swells. Also, due to its highly elastic nature, free standing mechanical structures can collapse. The vast difference of the Young's modulus between PDMS (360-870 kPa) and other inorganic materials like metals (gold- 79 GPa), leads to stress generation, buckling and cracking of inorganic films making electrical contacts formation difficult on PDMS.

2.1.4 Poly methyl meth acrylate (PMMA)

PMMA is a transparent low cost polymer with a very low auto-fluorescence over a wide spectral range. The polymer can be used as a masking material for patterning of devices and also as a structural material for the fabrication of low cost devices. The polymer can be patterned by a variety of methods, including: hot embossing, laser ablation, e-beam patterning and X-ray lithography. Unlike, silicon and glass which require high temperature and voltage for bonding; PMMA can be bonded at a lower temperature or with solvent assisted methods, giving it an advantage of a low thermal budget and a less expensive fabrication cost. PMMA has also been used as a mould material for fabrication of PDMS devices. PMMA has been widely used in the fabrication of microfluidic chips for DNA hybridization assays, antigen-antibody interactions and cell culture (Ni et al., 2009, Wang et al., 2003, Hashimoto et al., 2005, Wang et al., 2008, Hansberry and Clark, 2012). Suter et. al., have fabricated cantilevers of PMMA and magnetic composite of PMMA (Suter et al., 2011). Fung et. al.,

have demonstrated the fabrication of pressure sensors based on PMMA and Carbon nanotubes as the sensing elements (Fung et al., 2005). PMMA coatings have been used as a sensing layer for ammonia on optical fibers (Yu and Shiquan, 2011).

In spite of the above discussed advantages for polymeric devices, there are certain limitations also, which are associated with polymeric materials. Polymer surfaces require better control of surface chemistry than what is required in case of silicon or glass; they are also incompatible with organic solvents and high temperature processes (Anderson et al., 2000). Another property, which is a deterrent for sensitive optical detection in polymeric devices is that most of the polymers auto-fluoresce. Polymers are also more hydrophobic in nature, leading to surface adsorption of bio-molecules; unless they are coated with some bio-fouling agents. Therefore, proper attention needs to be given to the desired properties for the device in question and accordingly choose the material for fabrication.

2.2 Microfabrication Routes

MEMS/Bio-MEMS devices have been fabricated using the well-known micromachining techniques used in the integrated circuit domain, such as: photolithography, oxidation, sputtering, thin film deposition etc. The most widely used microfabrication processes are bulk micromachining and surface micromachining. These processes will be briefly discussed with a reference to fabrication of micro-cantilevers as shown in Figure 3. For a detailed discussion of the unit processes, readers can go through a number of available references on MEMS technology.

2.2.1 Bulk Micromachining

One of the oldest and the most mature technique for MEMS fabrication has been bulk micromachining. It is currently the most commercially viable method for fabrication of devices such as pressure sensors, accelerometers, microphones etc. The process involves selective etching/removal of bulk substrate material for the realization of MEMS devices. The etching away of bulk material can lead to the formation of MEMS components like beams, cantilevers, membranes etc. The removal of the bulk material can be achieved by wet/dry chemical methods or physical methods. Commonly used process is chemical wet/dry etching, wherein, a reactive chemical in wet or dry form etches away the unmasked regions of the bulk material. Chemical wet etching is favourable in cases where a high etch rate is required. Dry etch favours the formation of high aspect ratio structures with arbitrary geometries, which are not possible to achieve with the wet etching methods. Bulk micromachining methods are favourable for the fabrication of devices with an integrated electronics. The disadvantages associated with bulk micromachining is the control of etch depth with time across the substrate due to the variation in diffusion of etchant species, etchant ageing, loading effects and substrate thickness variation. In addition to this, the devices where electronics need not be associated with MEMS devices, bulk micromachining will not be cost effective.

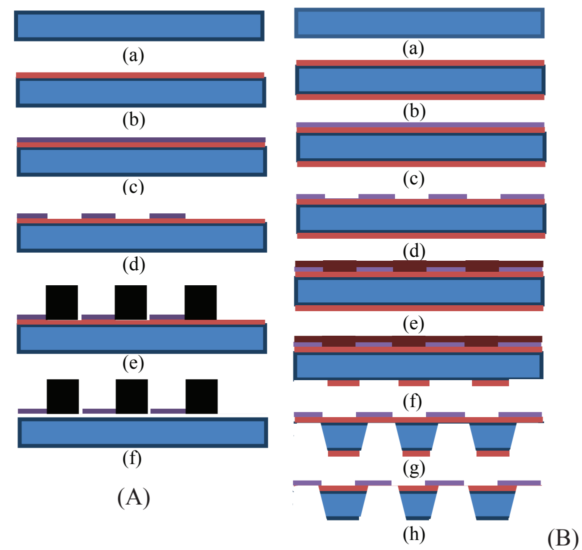

Figure 3: Basic Description of the Fabrication Processes (A) Surface micromachining: (a) Silicon wafer as substrate, (b) deposition of the sacrificial layer, typically Silicon dioxide, (c) Deposition or spin coat of the cantilever layer, (d) Patterning of active region of the cantilever, (e) Patterning of the anchor layer and (f) removal of the sacrificial layer for release of cantilevers (B) Bulk Micromachining-: (a) Double side polished

2.2.2 Surface Micromachining

Surface micromachining allows making structures on the surface of the substrate material and does not utilize the bulk of the substrate. It has been widely used since the beginning of 1980s. The process involves deposition and patterning of a mechanically supporting layer termed as “sacrificial layer” on the substrate, and subsequent deposition and patterning of thin films, the structural layers, on the sacrificial layer. The sacrificial layer is selectively etched on completion of the fabrication process, and the movable micromechanical devices are thus, released from the substrate. The advantage of surface micromachining lies in the fact that dimensions of structures can be several orders of magnitude smaller than that obtained with bulk micromachining, and the devices can be conveniently incorporated with the electronics. Two commercially available devices, which have been fabricated using the surface micromachining processes are the deformable mirror display from Texas Instruments, which use aluminium as the structural layer and photoresist as the sacrificial layer, and the gyroscope from Analog Devices, which uses poly-silicon as the structural layer and PSG as the sacrificial material (Ziaie et al., 2004).

Silicon wafer, (b) Oxidation, (c) Deposition or spin coat of the cantilever layer, (d) Patterning of active cantilever region, (e) Masking layer for top oxide, (f) Patterning and etching of bottom oxide, (g) Wet or dry etching of Silicon from bottom, and top oxide acts as the etch stop layer, (h) removal of the oxide to release the cantilevers.

Traditionally, integrated circuit fabrication processes have been used for MEMS devices realization. With an increase in know-how of microfabrication techniques, an introduction of new materials like polymers and ceramics, apart from silicon and glass and surface functionalization methods for Bio-MEMS; there has been an introduction of alternative techniques for the fabrication of such devices at a lower cost. Polymer devices essentially provide an advantage over the silicon devices for bio- and/or chemical applications due to their bio-compatibility, tuning capability of their surface groups for various applications, the feasibility of forming complex 3-D structures and cost effective fabrication processes. Commonly used alternative routes of fabrication of polymeric devices for the Rapid Prototyping are: hot embossing, soft lithography, stereo-lithography and laser micromachining.

2.2.3 Stereo-lithography

There has been a recent emergence of new fabrication technologies, which allow the realization of devices by 'layer by layer' approach. Stereo-lithography is one such process, which has evolved as a commercially viable process for rapid prototyping. It involves UV curing of the liquid polymer for achieving high aspect ratio structures. The process being a direct write from 3-D Computer Aided Design (CAD) models reduces the lead time of design to prototype from days to few hours. Wicker et. al., have demonstrated the application of stereo-lithography for the realization of devices for nerve regeneration and guided angiogenesis applications (Wicker et al., 2004). The technique has also been used for the preparation of moulds used in the preparation of implants. The limited number of commercially available resins to be used for stereo-lithography can be considered as the limitation of this technique (Melchels et al., 2010).

2.2.4 Hot Embossing

Hot Embossing is a comparatively faster and an inexpensive technique to replicate microstructures, especially microfluidic lab on chips in polymers. It involves the usage of thermoplastics as substrate for the transfer of microstructures from a mould, made in silicon or steel. In this process, the mould and the substrate (thermo-plastic) are heated together at a temperature above the glass transition temperature of the substrate with a controlled force applied for a given time duration. The assembly is then cooled below the glass transition temperature and subsequently, mould and substrate are de-embossed. Common plastics like polymethylmethacrylate (PMMA), polystyrene, Polyvinylchloride (PVC), polystyrene (PS) etc. have been used for hot embossing with an excellent reproducibility (Becker and Locascio, 2002).

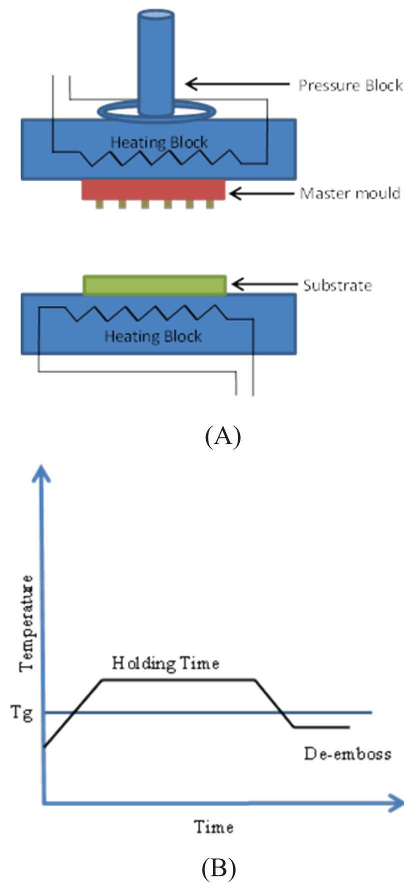

Figure 4: Schematic of Hot Embossing (A) Description of a typical system and (B) Temperature profile for the complete process. Tg= Glass transition temperature of the polymer at which it starts to change from amorphous to molten state.

2.2.5 Soft lithography

A popular method for fabrication of polymeric devices is soft lithography of moulding of elastomeric substrates like polydimethylsiloxane (PDMS) against a master mould. The process was introduced by Whitesides et al., for the fabrication of microfluidic devices (Zhao et al., 1997, Xia and Whitesides, 1998). The process involves formation of a master, either in photoresist by standard lithographic techniques or in metal by laser micromachining, and a liquid PDMS pre-polymer along with the curing agent is poured over the master and is heated. The PDMS pre-polymer, due to its low surface free energy, takes the shape of the master mould and on heating is cured (cross-linked) to form the negative relief of the master mould (Figure 5). The process does

not require any expensive equipment or clean room facilities and has an advantage of simplicity, lower cost and a replication accuracy of 10µm.

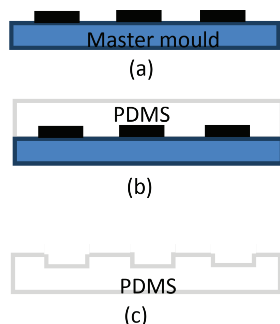

Figure 5: Soft lithography schematic (a) master mould is fabricated with lithography or rapid prototyping and is silanized for anti-stiction layer, (b) PDMS pre-polymer and curing agent (10:1) is mixed, de-gassed and poured on the master mould and heated on a hot plate, (c) negative relief in PDMS after peeling off.

3. Immobilization of biomolecules

The essential component of a biosensor is the formation of a compact layer of bio-recognition element in close proximity to the transducer, to which the target molecules bind subsequently. These molecules are usually nucleic acids, proteins (including antibodies, peptides etc.), carbohydrates or cells and are called as probe molecules. The immobilization of these molecules has a pivotal role in sensor fabrication because it affects sensor's limit of detection, specificity, reusability as well as reliability. The immobilization strategy of sensors with these molecules is that it must form stable, homogenous and reproducible layers on the surface, and also retain the activity of the probe molecules and its availability to the target molecule. The probe molecules can be bound by physical methods- adsorption, encapsulation or entrapment on the surface, or by chemical methods- covalently attached to the linker layer formed on the surface. Figure 6 gives the schematics of various immobilization methods.

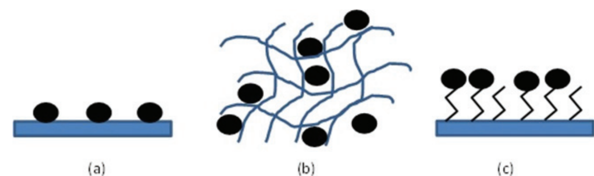

Figure 6: Schematic of different immobilization methods (a) Physical or non-specific adsorption, (b) Entrapment in a matrix or gel, (c) Covalent binding with linker molecules attached to surface.

Physical methods of immobilization of proteins or antibodies on the surface in most cases led to loss of activity of molecules. There are issues of orientation of the probe molecules such that the binding site for the target molecule is not always exposed to the surface, thus causing a loss of sensitivity. Changes in the environmental conditions such as pH, ionic strength, temperature etc., of the adsorbed proteins, may lead to detachment of bio-molecules from the surface. In case of entrapment of enzymes or cells in a matrix, there is a diffusion barrier limitation of the target molecules to the probe, leading to an increase in response time. Therefore, a linker layer on which probe molecules can be covalently attached is a better option. For covalent attachment of probe molecules, surface preparation steps are required, which can modify the surface to reactive functional groups which, in turn, can bind directly to probe or bind the probe molecules via a linker molecule. Once, the probe molecules are attached, it is essential to block the unreacted surface sites with anti-fouling molecules to minimize non-specific adsorption. Various strategies can be explored for covalent binding of probe molecules depending on the nature and chemical structure of the surface. The following section gives a brief description of immobilization on commonly used materials for biosensing. For a more detailed description, readers can refer to reviews on immobilization methods.

3.1 Immobilization on polymeric substrates

Covalent immobilization on polymer surfaces can be achieved by modifying the surface chemistries of the polymer to obtain functional groups like CHO, NH2, SH, COOH etc., which can be further used to bind proteins, DNA or cells. SU-8 has emerged as the most commonly used polymer in MEMS fabrication, owing to its photo-patternable property, attractive mechanical properties, and its inertness to various chemicals. Various research groups have explored different surface functionalization routes for the formation of active groups on surface. Joshi et. al., have reported the functionalization of SU-8 surfaces with active amino groups by following the wet silanization chemistry (Joshi et al., 2007b).

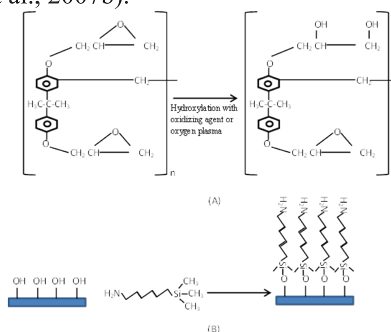

Figure 7: SU-8 functionalization with surface amino groups (a) hydroxylation reaction by oxygen plasma or strong oxidizing agents and (b) amino silanization reaction.

Other methods include the functionalization of SU-8 surface by grafting polymers (Gao et al., 2006, Wang et al., 2007), PEGylation of surface (Tao et al., 2008), glycine linker (Deepu et al., 2009), and silanization (Blagoi et al., 2008, Cao et al., 2011). Joshi et al., have also reported a unique dry method; wherein, ammonia gas is cracked on a hot filament surface in HWCVD system leading to formation of amino groups on SU-8 surface. This single step dry method not only reduces the number of steps for functionalization but; also, limits the substrate damage by harsh wet chemicals like strong oxidizing/hydrolysing agents (Joshi et al., 2007a). Polymers can be modified by grafting hydroxyl or amino groups by using oxygen or ammonia/nitrogen plasma treatment or UV light with/without ammonia atmosphere respectively (Park et al., 2002, Meyer-Plath et al., 2003).

3.2 Immobilization on gold

Gold is the most commonly used material for biosensors, due to its inert nature, good reflecting surface and good electrical properties. Thin gold films are also used in nano-mechanical sensors like cantilevers to provide asymmetric immobilization of biomolecules, where surface stress variations between opposite side of the cantilevers is studied and also for optical deflection measurements. Commonly used methods for the attachment of biological molecules at the surface of gold are through the formation of self-assembled monolayers of thiols, polyelectrolyte layers, Langmuir Blodgett films, biotin and protein-A chemistry and through conducting polymers. The high affinity of sulphur to gold is exploited to form Self assembled monolayers of thiols with various head groups like carboxylic acids, amino groups, esters and alcohols for the immobilization.

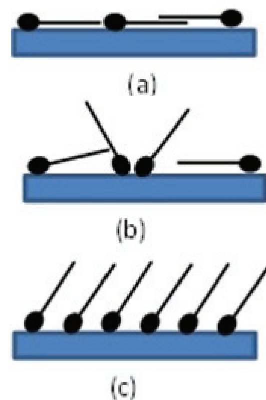

Figure 8: Schematic of self-assembly of thiol monolayers on gold (a) chemisorption of sulphur group, thiol on gold surface in lying down phase, (b) intermediate stage of stacking and (c) ordered standing up phase of thiol monolayers.

Bio-affinity, that is binding with protein-A or biotin-streptavidin conjugate has also been used by various research groups for oriented immobilization of antibodies.

3.3 Immobilization on silicon oxide and silicon nitride

Silicon oxide and silicon nitride are CMOS compatible materials and have been widely used for the fabrication of MEMS/Bio-MEMS structures. Cantilever or beam like structures and ISFETs/BIOFETs use silicon nitride/oxide as structural layers or dielectrics respectively. Therefore, immobilization strategies for binding biomolecules on these layers are of great interest. Silicon nitride films are usually non-porous and uncharged; hence, binding of biomolecules due to electrostatic binding or physical adsorption is ruled out. Hence, surface modification of these surfaces for covalent attachment of biomolecules is necessary (Diao et al., 2005). Commonly used method for modification of silicon oxide and nitride surfaces is by silanization with various head groups, like: NH2, COOH, CHO etc. (Diao et al., 2005, Hashim et al., 2013, Kale et al., 2009, Kurihara et al., 2012). Oxygen combines with plasma to form oxynitride and then an anhydrous silanization has also been reported (Joshi et al., 2004). A non plasma process is highly desirable due to the damage, which might occur in the suspended structures owing to the pressures in plasma process and damage to the substrate surface due to high energy particles present. Silicon nitride surfaces have also been treated with acrylic acid vapour at radio frequency plasma treatment to form carboxylic groups directly on the surface (Costa et al., 2012). Silicon oxide surface adsorbs water to form hydroxylated surfaces, which can further be modified by appropriate silane molecules in a similar fashion to silicon nitride.

4. Applications of Bio-MEMS

Biosensors on chip are relatively a new concept. Traditionally, any device which is capable of identifying (qualitatively or quantitatively) a biological signal or event can be labelled as a biosensor. A simple thermometer or stethoscopes are very basic and primitive examples of a biosensor. Biosensors can be broadly divided into two categories, invasive and non-invasive. Invasive sensors usually accompany therapeutic devices like pacemakers and are implanted inside the human body (Hitchcock and Sorenson, 2005, Yang, 2003). Recently many implantable devices have been reported, which can perform sensing and automatic wireless telemetry (Lei et al., 2006). A brief overview of recent advances in implantable sensors is given in the therapeutics section.

A huge array of non-invasive Bio-MEMS technologies and devices have been reported and developed. It becomes difficult to characterise the different technologies as many possible strategies exist.

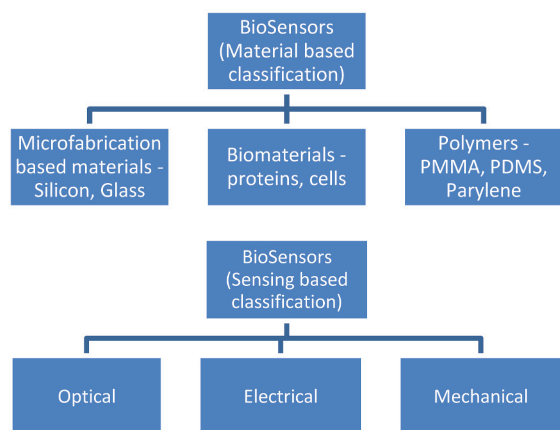

Figure 9: Classification of biosensors based on materials involved and sensing approach.

The classification based on sensing approach is elaborated in this review paper.

4.1 Biosensors based on optical sensing

Sensing of biochemical interactions, using change in optical properties (amplitude, polarization, resonance shift, and/or phase) is a key research area, which has vast applications in health care, environmental monitoring, biomedical research and pharmaceuticals. These sensors probe the surface in a non-destructive manner. Both label-free and fluorescence/chemiluminiscence techniques come under the ambit of optical biosensors. While labelled techniques are quite sensitive that it can even pin down a single molecule that is tagged with the target dye / protein; the process of tagging for detection is very complex and not cost effective. Thus, label-free techniques have an advantage over their counterparts in the fact that they are easy to reproduce and have easy detection methodology. Label free bio sensing/detection works mostly on the following principles:

4.1.1 Evanescent wave based biosensors

When a light beam travelling from an optically rarer medium to an optically denser medium is reflected off the interface, a small evanescent wave is injected into the optically denser medium. This evanescent wave is characterized by the exponential decay of electric field in the denser medium. The Evanescent wave based sensors use waveguides for transmission of the excitation and detection signals. Waveguides can be made of glass, quartz, optical fibers or polymers. These sensors work on the principle of total internal reflection due to the adjustment of refractive indices at the interface with the medium (Marazuela and Moreno-Bondi, 2002). A majority of evanescent biosensors are based on optical fibers (Leung et al., 2007). When molecules attach to the outer surface of the optical fiber, there is a change in the refractive index of the medium outside the core of the fiber, resulting in change in the intensity of light transmitted through the fiber. At the other end of the optical fiber, there is a photo-detector or photo-meter attached, which detects the change in the light intensity at the output end before and after the attachment of analyte molecules to receptors immobilized on the fiber. This change acts as a measure for the attachment of the analyte. These sensors have been widely used for the detection of pathogens (DeMarco et al., 1999, Ko and Grant, 2006, Geng et al., 2004) with a detection of 70cells/ml of E.coli as reported by Rijal et al. (Rijal et al., 2005). Clinical measurands like haemoglobin (Preejith et al., 2003), IgG (Hale et al., 1996, Tromberg et al., 1987), myoglobin and cardiac troponin I (Tang et al., 2006), progesterone (Tschmelak et al., 2004) and other such molecules of interest have also been quantified by using fiber optic sensors down to a concentration ranging from 1µg/ml to 1ng/ml. One of the most important consideration of these sensors is the necessity to immobilize recognition molecules close to the surface as the electric field decreases exponentially in the medium. The other limitations of these sensors is an interference of ambient light and background absorbance, fluorescence or Raman scattering of the fiber. The mass manufacturing of the evanescent wave fiber optic biosensors is tedious, as the technology for removing the cladding of the fibers is not mature enough for bulk production.

4.1.2 Interferometric biosensors

An interferometric sensing offers label free detection and relies on the splitting of a single coherent light source into two paths. One of the split light beam travels through the sample media, which is functionalized for the particular analyte of interest. Analyte binding causes a change in the refractive index along the optical path, resulting in a phase shift with respect to the non-functionalized reference path. This phase shift causes a difference in the interference pattern of the two beams (Myers and Lee, 2008). Different interferometers like the Mach-Zehnder interferometer, the Young's interferometer etc. are used for sensing. Ymeti et al. demonstrated an immunosensor to detect herpes simplex virus using the Young's interferometer configuration in a microfluidic chip and achieved femto-

molar range as limit of detection (Ymeti et al., 2007). Potential drawback of these interferometers is that a perfect matching of micromachined geometries and the material properties of waveguides is desired. Dmitry et al. (Dmitry et al., 2004) have shown the detection of streptavidin-biotin and protein-IgG in a PDMS microfluidic channel by using the back scattering interferometer.

4.1.3 Surface plasmon resonance (SPR)

The Surface plasmon resonance biosensor was first demonstrated by Liedberg et al. in 1983 (Liedberg et al., 1983). The Surface plasmon resonance phenomenon refers to the optical excitation of the surface plasmon oscillations on a thin metal layer on a dielectric by the evanescent waves leaking into the metal, during the reflection of light at this metal-dielectric interface. This surface plasmon resonance is characterized by a sharp absorption peak when the wave vector of the incident light matches to that of the plasmon resonance. This absorption peak is sensitive to the refractive index at the metal/ liquid interface and hence, becomes a powerful tool for characterization of reactions in real time. When a ligand binds to the surface of the metal, there is a change in the resonant angle, resonant wavelength, resonant intensity, or phase, which is measured by a laser beam. Many commercial SPR systems are available for immunosensing and DNA hybridization, most notably, the Biacore from GE healthcare and the Spreeta from Sensata. Waswa et al., (Waswa et al., 2007) demonstrated the use of Spreeta for the immunological detection of E.coli O157:H7 in milk, juices and beef extracts to a limit of 100 colonies/ml. SPR biosensors have been applied for the past few years for the detection of proteins at pico-molar to nano-molar ranges, DNA molecules at femto-molar levels with the aid of gold nanoparticles and for environmental monitoring and food safety (Fan et al., 2008).

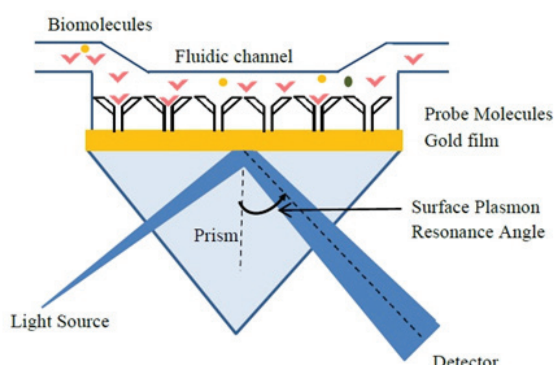

Figure 10: Principle of the surface plasmon resonance.

The advantage of SPR lies in the fact that real time monitoring of the reaction can take place, which helps in studying the reaction kinetics. It can also handle turbid or coloured samples, which are a limitation for other optical methods. The main challenge in SPR is its limitation of sensitivity when low molecular weight analytes are to be measured. These molecules, when attached to the surface, do not produce a substantial change in the refractive index. The sensitivity of the measurement; also, depends on the medium containing reagents and temperature of the surrounding. Multiplexing bio-molecular interactions with SPR still remains a challenge because of the complex fluidics and instrumentation involved.

4.1.4 Photonic crystal biosensor

Photonic crystals are periodic arrays of material with a different dielectric constant, whose photonic transmission or reflection modes can be engineered. The periodicity of the array is of the order of the wavelength of interest. The Photonic biosensors use this property to perform label free bio-sensing. Binding of biomolecules to this structure will alter this response, which is used as an index to interrogate a bio-molecular interaction. There is a resonant peak/dip at a particular wavelength, characteristic of the periodicity and symmetry of the dielectric material. When a particular molecule is attached to the surface, there is a shift in the resonant peak/dip indicating binding. This shift acts as a marker that gives the sensitivity to this class of biosensor.

Optical detection may have a cost advantage over other sensors, because unlike electrochemical detection, electrodes do not have to be integrated on the disposable device. Using the advantage of commercial CCD or CMOS image sensors, multiplexing could be achieved at a pixel scale. However, the optical detection generally requires expensive hardware which is difficult to miniaturize, and it suffers at lower length scales. The shorter optical path lengths through the sample lead to a reduced sensitivity, and lower surface to volume ratios lead to an increased noise from a non-specific adsorption to chamber walls.

4.2 Biosensors based on electrical/electrochemical sensing:

Electrochemical sensors are by far the most commonly reported sensors in the literature and have also dominated the biosensor market for years. The first biosensor, glucose biosensor based on the Clark oxygen electrode, was developed as an electrochemical biocatalytic sensor, which used glucose oxidase to measure the glucose content. These sensors combine the analytical advantages offered by electrochemistry

and the specificity of biological recognition process, thus making the technique quite sensitive and specific.

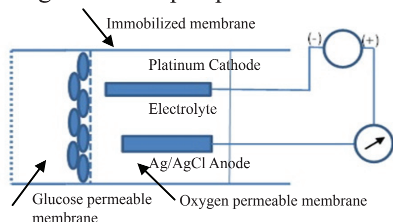

Figure 11: Glucose biosensor based on the Clarke oxygen sensor (Li, 2007). The enzyme, glucose oxidase, immobilized on the membrane, catalyses the formation of products by metabolizing glucose in the sample. There is an exchange of oxygen and electrons through the membrane leading to the flow of current, which acts as the quantification of the glucose present in the sample.

Electrochemical sensors are based on the electrochemical species consumed and/or generated during a biological and chemical interaction process of a biologically active substance and substrate. This electro-chemical reaction causes some change in the electrical properties of the solution, which can be used as a measuring parameter. Electrochemical biosensors are fast, simple, low-cost, more amenable to miniaturization with a compatible sensitive instrumentation existing and can be operated in turbid and/or coloured media. These advantages have made electrochemical biosensors the most suitable choice for the design of portable bioanalytical devices. Electrochemical sensors can be classified as amperometric, potentiometric, conductometric or impedimetric, depending on the signal (potential or current) applied and the technique used to measure change.

4.2.1 Amperometric biosensors

Amperometric sensors work on the principle of measuring current, generated due to the oxidation or reduction of an electroactive species, while a constant potential is maintained. The current generated is directly proportional to the consumption or generation of the electroactive species in the vicinity of the bio-receptor layer. The first biosensor, the glucose clark sensor was based on amperometry. These sensors have an advantage of simplicity, an ease of production and low cost of devices and instruments. Moreover, this method is insensitive to chromogens and sample turbidity, which allows whole blood samples to be analysed.
Amperometric sensors have been shown to detect concentrations of the analyte in picomolar range and have thus, resulted in a wide exploration of these sensors (Schöning et al., 2003, Hervás Pérez et al., 2006, Türkarslan et al., 2009, Liu et al., 2007, Madaras et al., 1996, Schoning et al., 2005). The measurement system for these sensors uses three electrodes: the working electrode where the desired reaction occurs, the reference electrode to maintain a constant potential at the working electrode and the counter electrode to complete the circuit. Amperometric biosensors are of three different types, depending on the electron transfer mechanism involved.

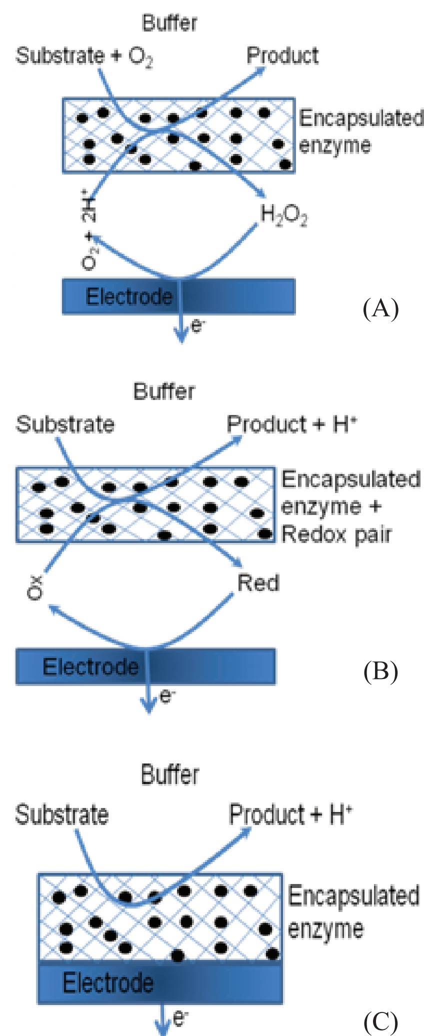

Figure 12: Types of amperometric biosensors. (a) First generation electrode utilizes the enzyme-substrate reaction, and the product is used as measurand (b) Second generation electrode includes a redox mediator to transfer the electrons to electrode and (c) Third generation electrodes utilize the reaction produced electrons directly.

The limitation of amperometric sensors is that only electrochemically active compounds can be detected. Therefore, antigen-antibody or other protein detection can take place only when one or more of the component taking part in the reaction is labelled. Secondly, the reaction taking place at the surface of the electrode can cause electrode fouling due to the accumulation of

products or due to the adsorption of proteins or other cellular components.

4.2.2 Potentiometric biosensors

In the potentiometric sensors, the change in the potential between the working and the reference electrode, due to surface charge accumulation with time is measured keeping a zero current condition. The potential changes are governed by the Nernst equation and are logarithmically proportional to specific ion activity. The transducer for these sensors can be an ion sensitive electrode (ISE), which has thin films or selective membranes as recognition elements or an ion-sensitive field effect transistor (ISFETs), which has enzymes or proteins attached to the gate surface. ISFETs are based upon the modulation of gate potential by pH or ionic change near the surface. The first potentiometric biosensor was introduced in 1969 by Guilbault and Montalvo (Guilbault and Montalvo Jr, 1969) by coupling an ion selective electrode with the urease enzyme to detect urea. Later, these sensors have been used for the detection of glucose, amino acids and penicillin among other molecules (Nilsson et al., 1973, Nagy et al., 1973, Liao et al., 2007).

These sensors are less sensitive than the amperometric sensors and have a detection range of millimolar concentration of analytes. This technique; also, suffers from the possibility of interferences due to changes in the ionic strengths of the analyte medium and non-specific reactions taking place on the surface. At times, the change in the potential due to the interaction (antigen-antibody binding) on the surface is too small, of the order of 1-5mV, to be detected reliably.

4.2.3 Conductometric biosensors

The principle of these sensors is based on the change in the conductivity of the medium as a result of binding of receptor to its complementary analyte. Watson et al. were among the first to report a conductometric based enzyme electrode (Watson et al., 1988). The electrodes can be arranged laterally with the selective layer in between them. The measurement in this configuration is done in DC mode and is suitable to gases or non-conducting medium. The other configuration has been adapted from an electrochemical cell. In this configuration, the impedance is measured perpendicular to the interface between the selective layer and the conductive ionic sample.

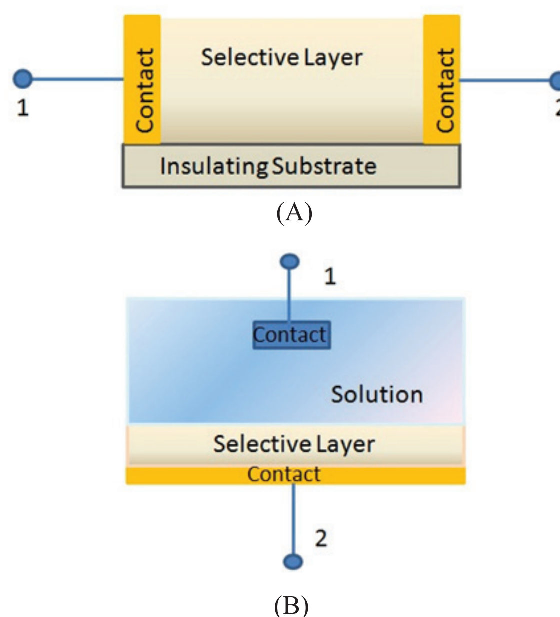

Figure 13: Cross-section of different configurations of the conductometric sensor (figure not to scale). (a) planar and (b) parallel configuration of electrodes. In the planar configuration, it is the inter-electrode changes which are measured, while in the parallel surface or near to surface change is what which pre-dominate.

Conductometric sensors have been used for environmental monitoring (Wang et al., 2006, Jaffrezic-Renault and Dzyadevych, 2008) and clinical diagnostics (Okafor et al., 2008, Mikkelsen and Rechnitz, 1989, Kanungo et al., 2002). The bulk liquid conductivity changes are measured with this technique, thereby reducing its specificity, as similar ions or physical adsorptions can; also, lead to such conductivity changes.

4.2.4 Impedimetric immunosensors

Electrochemical impedance spectroscopy is a rapidly growing transduction method. It can be used for the characterization of electrodes functionalized with biomolecules and the transformations, which follow due to the binding of the corresponding antigen at the electrode-electrolyte interface. This method gives a detailed information on the resistance and/or capacitance changes occurring at the electrode surface. A small perturbation is applied to the system, usually in the form of sinusoidal voltage and the current generated is thereby measured.

Capacitive or impedimetric sensors are among the type of sensors, which can easily be integrated along with the micro-channels in a miniaturized format and still be sensitive enough to detect molecules in a

sample volume of nano- or micro-litres. Since the first capacitive transducer for liquids was reported in 1986, technology has witnessed continuous up-gradations to fabricate such sensors for use in health care. The sensing is based on the application of an AC signal excitation to the electrode where reaction occurs, and measuring the corresponding output signal in terms of amplitude and phase change.

This detection can be made either in contact or in contactless configuration. A contactless electrode makes fabrication of such sensors easy and also eliminates the interferences due to high voltages applied, or the fouling of electrodes due to the reactions involved in the electrolyte. Contactless configuration can be achieved by fabricating the electrodes outside the channel wall, or they can be integrated within the channel with an insulating layer of self-assembled monolayers or oxide formed on the metal surface.

Capacitive immunosensors based on the Electrolyte-Insulator-Semiconductor (EIS) structures have been the subject of research for the past decade. The specific interaction between an antibody immobilized on a dielectric and the corresponding antigen is reflected in the change in the capacitance of the structure. EIS sensors have been used for the detection of molecules of clinical interest (Betty et al., 2004, Prasad and Lal, 1999, Gardies et al., 1989).

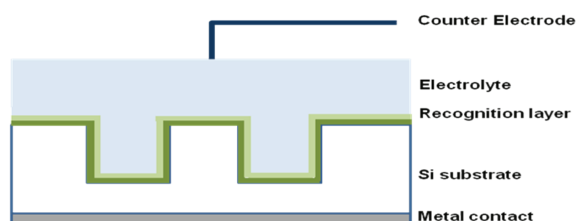

Figure 14: Schematic of an electrolyte insulator semi-conductor sensor.

These sensors can also be modified to be made as metal insulator electrolyte metal (MIEM) capacitors. Due to their low cost of manufacturing, low power consumption, less complicated instrumentation, ruggedness and ease of miniaturization; electrochemical biosensors hold the promise for applications where minimizing size with an ease of use and cost is important, as is the case of point of care (POC) diagnostics.

4.3 Biosensors based on mechanical detection:

One of the commonly explored ways for mechanical detection of antibody-antigen reactions is by employing micromachined cantilevers. The key principle exploited in detection using cantilevers is the characterization of deflection of the cantilever due to the bio-capture event. A basic overview of the functionalization of surfaces has been discussed in Section 3. Once these functionalized cantilevers are exposed to the target bio- or chemical species, a volumetric and/or mass change occurs, leading to the deflection of cantilevers. This inherent property of cantilever deflection offers the advantage of avoiding external labelling and detection. This deflection can be characterised in two ways, electrical and optical.

4.3.1 Optical detection

The principle of optical detection is borrowed from the Atomic Force Microscopy. A laser is pointed at the tip of the cantilever. The deflection of the cantilever is measured by using the laser spot, reflected from the surface, using a position sensitive detector.

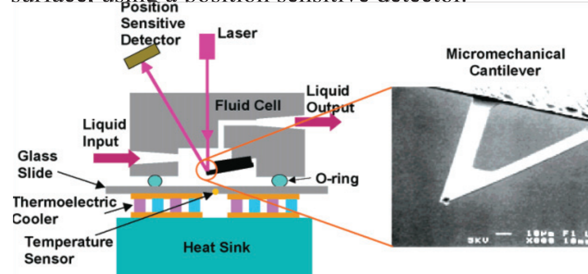

Figure 15: Experimental setup and SEM image of the micro-cantilever (Hansen et al., 2001).

Reprinted with permission from Hansen et al., 2001.
Copyright (2001) American Chemical Society.

Although the optical detection has the advantages of achieving very high sensitivity; its implementation remains very complicated and expensive, making it impractical for a portable lab on a chip application.

4.3.2 Electrical detection

Microcantilevers can be driven in two basic modes: static and dynamic. The sensing approaches are briefly explained below. For an in depth reading, readers are referred to an excellent review paper by Tamayo et al. (Tamayo et al., 2013).

Electrical sensing – Static mode

In static mode, the surface stress on the cantilever leads to a static deflection. This deflection can be measured either optically or electrically. The optical method was briefly explained above. Many approaches for electrical detection have been employed, for instance, a layer of gold was deposited on the surface of the cantilever to function as a strain gauge (Nordström et al., 2008). Another method employed is to embed a piezoresistive layer (of PolySi, SU8 – Carbon black composite) within the cantilever, and to use an electrical readout.

Cantilever – Static mode sensing theory

When a bio-chemical target is captured on the cantilever surface, a surface stress is induced causing bending of the cantilever. The bending of the cantilever is actually a result of the non-active surface trying to equalise the surface energy variations occurring on the active surface, through elastic expansions or contractions (Ibach, 1994). The Stoney's equation gives the linear relationship between the surface stress (Δs) and the curvature change (Δc):

$$\Delta c = 6 \frac{1 - \nu}{E h^2 \Delta s}$$

where ***E*** is the Young's Modulus, *ν* is the Poisson's ratio and h is the thickness of the cantilever beam. The equation also indicates the design parameters one needs to consider for obtaining higher sensitivity for a given surface stress. Traditionally, inorganic cantilevers made of silicon and/or silicon nitride have been used. Typically, the Young's Modulus of Silicon is around 150GPa and those of organic polymeric platforms are around 1-5GPa. This suggests that moving to organic cantilevers can increase the sensitivity by at least an order of magnitude, when compared to silicon based cantilevers. A sensitivity gain by a factor of six was demonstrated by Calleja et al. by using polymeric SU-8 cantilevers (Calleja et al., 2005).

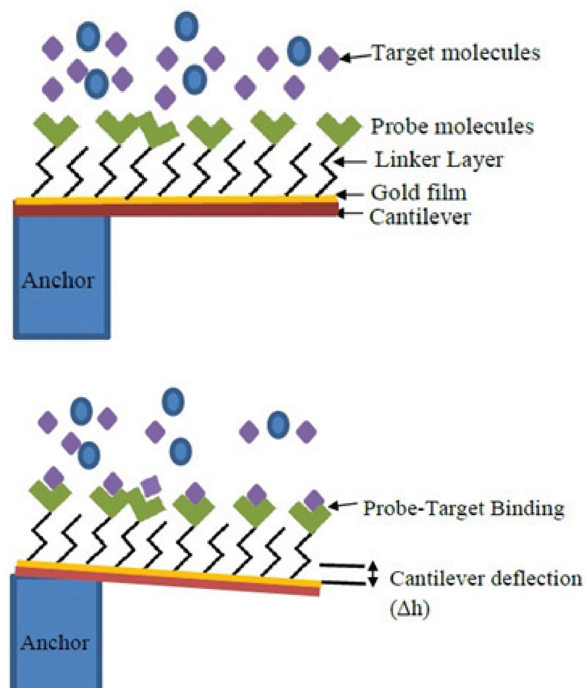

Figure 16: Bio-molecular interactions on one surface of the cantilever beam resulting in surface stress, which causes the beam to bend.

Piezoresistive readout

To enable electrical readout of cantilever deflection, a piezoresistive layer is embedded into the cantilever. The fabrication and structure of such multi-layered cantilevers has been discussed in Section 2.2. These cantilevers can be employed in a simple wheatstone bridge structure, and the change in the resistance (ΔR) of the piezoresistor can be measured. This ΔR directly correlates to the surface stress, as given by the following equation (Seena et al., 2009a):

$$\frac{\Delta R}{R} = 3K \frac{\left(1 - \frac{Lpiezo}{2L}\right) d}{L^2} Z$$

where ***K*** is the gauge factor of the piezoresistive material, L_{piezo} is the length of the piezoresistive layer, L is the length of the cantilever, d is the distance from the neutral axis and Z is the deflection.

4.3.3 Applications of micro-cantilevers in sensing

Micro-cantilevers, owing to their physical characteristics provide an attractive solution to sensitive and rapid detections (Fritz et al., 2000). Such advantages are highly sought out for point of care medical diagnostics. Many groups across the world are involved in an active research, some leading to prototypes. For instance Rao et al.(Seena et al., 2009b) have been exploring the applications of polymer cantilevers for biochemical detection with a special focus on cardiac diagnostics. Polymer cantilevers (for instance SU-8, young's modulus – 4GPa), owing to their lower young's modulus, have an intrinsic advantage of greater sensitivity over traditional inorganic cantilevers (SiO2, young's modulus – 70GPa). This advantage enables detection in a low ppm or high ppb concentration levels. For instance, Troponin I and Troponin T are the most common biomarkers for cardiovascular diseases, and they exhibit elevated detection levels of 10-100ppb.

Depending on the material of cantilevers, strategies for fabrication and bio-functionalization change. A brief overview of fabrication and surface modification strategies is given in some section. Usually, the first step in any bio-functionalization study is immunofluorescence. The cantilevers are functionalised with fluorescence tagged antibodies, and the efficacy of the protocol is tested. Such studies and results have been reported by various research groups (Kale et al., 2009).
For an effective sensing, an asymmetric immobilization

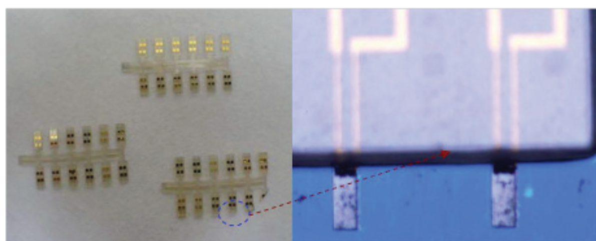

Figure 17: Optical micrograph of polymeric cantilevers (Seena et al., 2009b).

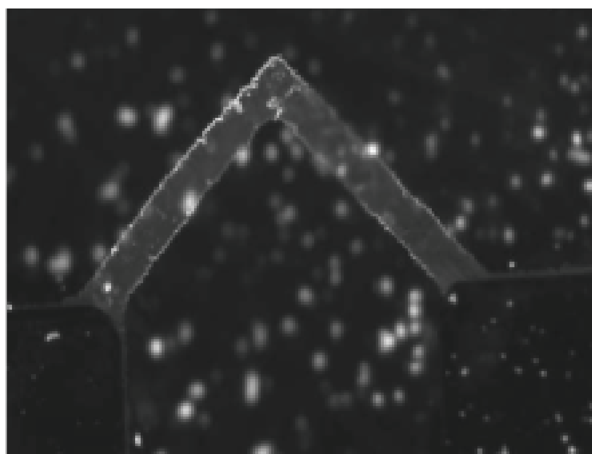

Figure 18: Fluorescence microscopic image of FITC (green label) tagged antibodies on nitride cantilever (Kale et al., 2009).

is important. If the antibodies get immobilized on both the surfaces (top and bottom) of the cantilever, the stress generated will cancel each other. Thus, it is important that antibodies are immobilized only on one surface. One effective strategy to achieve an asymmetric immobilization is to sputter gold on one side, and perform silanization chemistry on the other side. This strategy was employed and reported by Joshi et al.(Joshi et al., 2010). Readers are referred to the same paper for further details regarding the protocol and some initial bio-sensing results.

Apart from bio-sensing, the microcantilevers have been found very effective for chemical sensing applications. Fe(III)Porphyrin coated micro-cantilevers have been found selective to carbon monoxide (CO) (Reddy et al., 2012). Benzimidazole-functionalized calix[4]arene receptor coated cantilevers have been found to be excellent receptors for TNT (trinitrotoluene) (Kandpal et al., 2013). Polyaniline (PANI) coated microcantilevers have been found to be very effective for humidity and soil moisture detection (Patil et al., 2014). Readers are encouraged to refer to all the respective papers for further details.

4.4 Microfluidics

A miniaturized analytical system like a Lab-on-a-chip or a micro- Total Analysis System (μ-TAS) includes at least separation and detection modules integrated on a chip for analyzing components present in samples. Additional sample pre-treatment, filtering or derivatization modules can also be integrated. During a chemical or biological analysis, separation of the analytes from each other and from the matrix constituents, which can interfere with the detection is an important aspect. In earlier times, it was done by separation methods using a large amount of sample as well as reagents. Off late, smaller systems which require miniscule amount of reagents and samples have been developed. The motivation behind developing such systems is not just the reduced amount of reagents or samples, but also reduced lab space, increased separation speed and efficiency. The typical dimensions of the microstructure are in the range of few micrometers to several millimetres in length and width and between 100s of nanometers to 100 μm in depth.

This concept of μ-TAS, came into existence as early as 1970's when Terry et al. miniaturized a gas chromatography system, and integrated the complete system on a silicon wafer (Terry et al., 1979). Incidentally, this work went unnoticed, until Manz et al. reported the fabrication of a μ-TAS system in 1990s. This discovery resulted in an avalanche of developments by different research groups towards the making of planar microfluidic systems on glass or quartz (Becker and Gaertner, 2001). A lab-on-a-chip technology is different from the microarray technology as it incorporates the multiple functionalities of a biochemical analysis. These functionalities are interconnected through microchannels, which constitute a key part in lab-on-chips.

The microdevices work in a regime where small volumes of samples are sufficient for detection. This property of microdevices is a boon for diagnostic systems where either the sample volumes are less or the sample is rare, expensive or hazardous. This includes the in vivo studies of the neurotransmitter release and other clinical applications. It is known that the number of molecules is sparse in the case of infectious diseases. Therefore, the use of miniaturized devices would be advantageous in such cases; especially, when there is a need of disease screening on-site as in the case of an epidemic or blood donation (St-Louis, 2000), food pathogens, environmental threats (Ohira et al., 2002) or a bioterrorism attack (Wang et al., 2002a, Wang et al., 2002b).

Microdevices are also useful in the case of more complex processes like polymerase chain reaction for DNA fingerprinting or genome analysis, clinical diagnostics (Kricka and Wilding, 1996, A. Van Den Berg, 1995, Lauks, 1998, Erickson and Wilding, 1993, Dempsey et al., 1997), drug screening (Effenhauser et al., 1997), cell studies and handling (Carlson et

al., 1998, Li and Harrison, 1997) and environmental monitoring (van den Berg et al., 1993); as additional pre-treatment, filtering and derivatization steps can also be included on the same chip (Findlay et al., 1993).

The main advantages of a lab-on-a-chip as compared to the conventional laboratory assays, include but are not limited to (a) reduced sample and reagent consumption and small amount of waste, which can be contained in the disposable device itself, (b) higher sensitivity, (c) shorter analysis time, (d) improved throughput by processing several assays in parallel (e) portability that allows in situ and real time analysis (f) low cost as they can be mass produced, (g) minimization of cross contamination, (h) energy efficient, (i) increased safety and reliability by having less external interconnects between different parts and (j) disposability due to low cost and mass production. The microfabrication techniques; also, allows to fabricate highly complex devices with a high degree of functionality on the same platform, making complex analysis simpler to perform (Selvaganapathy et al., 2001, Studer et al., 2002). In addition to the above mentioned advantages miniaturization; also, improves the molecular diffusion and heat transport without changing the nature of molecular reactions due to the increased surface area to volume ratio (Dittrich and Manz, 2006). All the above mentioned advantages make microdevices a powerful packaged device, which covers all aspects of performance and price factors. The radical change from the laboratory equipment and methods to miniaturized devices used in life sciences for drug discovery, clinical diagnostics, and analytical chemistry would open novel markets and generate new business opportunities. Investment in diagnostics and prevention can become more cost effective than treatment.

5. Conclusions

Last couple of decades have shown considerable progress in the microfabrication technology. As a result, new materials and complicated structures are now being realised, which have enabled exciting applications. Especially in the field of Bio-MEMS, many commercial applications for lab on chip diagnostic systems and microfluidics have emerged. Some of the basic strategies were mentioned above. The interdisciplinary nature of any Bio-MEMS sensor was highlighted, demonstrating the challenges and also the outcomes of such an effort. As advances are continuously being made in MEMS, optics and also in the field of medicine, more exciting research outcomes are anticipated. For instance, an improved understanding of DNA hybridization has led to the research and development on DNA hybridization sensors for cancer detection. An exciting discovery in any of the disciplines acts as a catalyst for research in wider array of applications, or an improvement in current ones. Hence, this is a continuously evolving field. Some of the exciting current and future research directions are the applications of Bio-MEMS sensors. field. Some of the exciting current and future research directions are the applications of Bio-MEMS sensors in robot assisted minimally invasive surgeries, targeted drug delivery and therapeutics. There is also active research exploring methods to integrate diagnostics and an immediate drug delivery. Special polymer materials have been developed that expand when exposed to certain conditions and release drugs (Schmaljohann, 2006).

In this paper, it was attempted to initiate the reader in very brief, some of the existing strategies for MEMS fabrication and bio-functionalization. Some applications were discussed. However, it is also acknowledged that this paper offers a very brief and short introduction to Bio-MEMS, which has now progressed to innumerable domains and applications. For a more elaborate reading, readers are referred to this excellent book (Saliterman, 2006), where the author goes into much more detail in many aspects, and also enlists various exciting possibilities emerging currently.

References

Van den Berg A. & Bergveld P., 1995. Micro total analysis systems, London, Kluwer Academic Publishers.

Abushagur, A. A., Arsad, N., Reaz, M. I. & Bakar, A. 2014. Advances in bio-tactile sensors for minimally invasive surgery using the fibre bragg grating force sensor technique: A survey. Sensors, Vol.14, pp.6633-6665.

Aguirregabiria, M., Blanco, F., Berganzo, J., Ruano, J., Aranburu, I., Garcia, J. & Mayora, K. 2004. Novel su8 multilayer technology based on succesive cmos compatible adhesive bonding and kapton releasing steps for multilevel microfluidic devices. SPECIAL PUBLICATION-ROYAL SOCIETY OF CHEMISTRY, Vol.297, pp.49-51.

Anderson, J. R., Chiu, D. T., Wu, H., Schueller, O. J. & Whitesides, G. M. 2000. Fabrication of microfluidic systems in poly (dimethylsiloxane). Electrophoresis, Vol.21, pp.27-40.

Arscott, S. 2014. Su-8 as a material for lab-on-a-chip-based mass spectrometry. Lab on a Chip, Vol.14, pp.3668-3689.

Bashir, R. 2004. Biomems: State-of-the-art in detection, opportunities and prospects. Advanced drug delivery reviews, Vol.56, pp.1565-1586.

Becker, H. & Gaertner, C. 2001. Polymer based micro-reactors. Reviews in Molecular

Biotechnology, Vol.82, pp.89-99.

Becker, H. & Locascio, L. E. 2002. Polymer microfluidic devices. Talanta, Vol.56, pp.267-287. Betty, C., Lal, R., Sharma, D., Yakhmi, J. & Mittal, J. 2004. Macroporous silicon based capacitive affinity sensor--fabrication and electrochemical studies. Sensors and Actuators B: Chemical, Vol.97, pp.334-343.

Bilenberg, B., Nielsen, T., Clausen, B. & Kristensen, A. 2004. Pmma to su-8 bonding for polymer based lab-on-a-chip systems with integrated optics. Journal of Micromechanics and Microengineering, Vol.14, pp.814.

Blagoi, G., Keller, S., Johansson, A., Boisen, A. & Dufva, M. 2008. Functionalization of SU-8 photoresist surfaces with igg proteins. Applied Surface Science, Vol.255, pp.2896-2902.

Calleja, M., Nordström, M., Álvarez, M., Tamayo, J., Lechuga, L. M. & Boisen, A. 2005. Highly sensitive polymer-based cantilever-sensors for DNA detection. Ultramicroscopy, Vol.105, pp.215-222.

Cao, C., Birtwell, S. W., Høgberg, J., Wolff, A., Morgan, H. & Bang, D. D. Surface modification of photoresist su-8 for low autofluo-rescence and bioanalytical applications. 15th International Conference on Miniaturized Systems for Chemistry and Life Sciences, 2011. pp.1161-1163.

Carlson, R. H., Gabel, C., Chan, S. & Austin, R. H. 1998. Activation and sorting of human white blood cells. Biomedical Microdevices, Vol.1, pp.39-47.

Costa, L. T., Vilani, C., Peripolli, S., Stavale, F., Legnani, C. & Achete, C. A. 2012. Direct immobilization of avidin protein on afm tip functionalized by acrylic acid vapor at rf plasma. Journal of Molecular Recognition, Vol.25, pp.256-261.

Deepu, A., Sai, V. & Mukherji, S. 2009. Simple surface modification techniques for immobilization of biomolecules on su-8. Journal of Materials Science: Materials in Medicine, Vol.20, pp.25-28.

Demarco, D. R., Saaski, E. W., Mccrae, D. A. & Lim, D. V. 1999. Rapid detection of escherichia coli o157: H7 in ground beef using a fiber-optic biosensor. Journal of Food Protection&# 174;, Vol.62, pp.711-716.

Dempsey, E., Diamond, D., Smyth, M. R., Urban, G., Jobst, G., Moser, I., Verpoorte, E. M. J., Manz, A., Michael Widmer, H. & Rabenstein, K. 1997. Design and development of a miniaturised total chemical analysis system for on-line lactate and glucose monitoring in biological samples. Analytica chimica acta, Vol.346, pp.341-349.

Desai, T., Ferrari, M. & Bhatia, S. N. 2007. Biomems and biomedical nanotechnology: Volume iii: Therapeutic micro/nanotechnology, Springer.

Diao, J., Ren, D., Engstrom, J. R. & Lee, K. H. 2005. A surface modification strategy on silicon nitride for developing biosensors. Analytical biochemistry, Vol.343, pp.322-328.

Dittrich, P. S. & Manz, A. 2006. Lab-on-a-chip: Microfluidics in drug discovery. Nature Reviews Drug Discovery, Vol.5, pp.210-218.

Dmitry, A., Swinney, K. & Bornhop, D. J. 2004. Label-free molecular interaction determinations with nanoscale interferometry. Journal of the American Chemical Society, Vol.126, pp.16659-16664.

Effenhauser, C. S., Bruin, G. J. M. & Paulus, A. 1997. Integrated chip-based capillary electrophoresis. Electrophoresis, Vol.18, pp.2203-2213.

Erickson, K. A. & Wilding, P. 1993. Evaluation of a novel point-of-care system, the i-stat portable clinical analyzer. Clinical chemistry, Vol.39, pp.283.

Fan, X., White, I. M., Shopova, S. I., Zhu, H., Suter, J. D. & Sun, Y. 2008. Sensitive optical biosensors for unlabeled targets: A review. Analytica chimica acta, Vol.620, pp.8-26.

Findlay, J. B., Atwood, S., Bergmeyer, L., Chemelli, J., Christy, K., Cummins, T., Donish, W., Ekeze, T., Falvo, J. & Patterson, D. 1993. Automated closed-vessel system for in vitro diagnostics based on polymerase chain reaction. Clinical chemistry, Vol.39, pp.1927.

Fritz, J., Baller, M., Lang, H., Rothuizen, H., Vettiger, P., Meyer, E., Güntherodt, H.-J., Gerber, C. & Gimzewski, J. 2000. Translating biomolecular recognition into nanomechanics. Science, Vol.288, pp.316-318.

Fung, C. K., Zhang, M. Q., Chan, R. H. & Li, W. J. A pmma-based micro pressure sensor chip using carbon nanotubes as sensing elements. Micro Electro Mechanical Systems, 2005. MEMS 2005. 18th IEEE International Conference on, 2005. IEEE, pp.251-254.

Gao, Z., Henthorn, D. B. & Kim, C.-S. Surface modification of su-8 by photografting of functional polymers for lab-on-a-chip applications. Sensors, 2006. 5th IEEE Conference on, 2006. IEEE, pp.121-123.

Gardies, F., Martelet, C., Colin, B. & Mandrand, B. 1989. Feasibility of an immunosensor based upon capacitance measurements. Sensors and Actuators, Vol.17, pp.461-464.

Geng, T., Morgan, M. T. & Bhunia, A. K. 2004. Detection of low levels of listeria monocytogenes cells by using a fiber-optic immunosensor. Applied and environmental microbiology, Vol.70, pp.6138-6146.

Guilbault, G. G. & Montalvo Jr, J. G. 1969. Urea-specific enzyme electrode. Journal of the American Chemical Society, Vol.91, pp.2164-2165.

Hale, Z., Payner S, F. & Lowem M, C. 1996. The single mode tapered optical fibre loop immunosensor. Biosensors and Bioelectronics, Vol.11, pp.137-148.

Hansberry, D. & Clark, P. Quantum dot-linked immunoassay on a pmma-based microfluidic device for biomarker detection. Bioengineering Conference (NEBEC), 2012 38th Annual Northeast, 2012. IEEE, pp.91-92.

Hansen, K. M., Ji, H.-F., Wu, G., Datar, R., Cote, R., Majumdar, A. & Thundat, T. 2001. Cantilever-based optical deflection assay for discrimination of DNA single-nucleotide mismatches. Analytical Chemistry, Vol.73, pp.1567-1571.

Hashim, U., Chong, S. W. & Liu, W.-W. 2013. Fabrication of silicon nitride ion sensitive field-effect transistor for ph measurement and DNA immobilization/hybridization. Journal of Nanomaterials, Vol.2013, pp.100.

Hashimoto, M., Hupert, M. L., Murphy, M. C., Soper, S. A., Cheng, Y.-W. & Barany, F. 2005. Ligase detection reaction/hybridization assays using three-dimensional microfluidic networks for the detection of low-abundant DNA point mutations. Analytical chemistry, Vol.77, pp.3243-3255.

Hervás Pérez, J., Sánchez-Paniagua López, M., López-Cabarcos, E. & López-Ruiz, B. 2006. Amperometric tyrosinase biosensor based on polyacrylamide microgels. Biosensors and Bioelectronics, Vol.22, pp.429-439.

Hitchcock, R. W. & Sorenson, J. L. 2005. Implantable biosensor system, apparatus and method. Google Patents.

Ibach, H. 1994. Adsorbate-induced surface stress. Journal of Vacuum Science & Technology A, Vol.12, pp.2240-2245.

Jaffrezic-Renault, N. & Dzyadevych, S. V. 2008. Conductometric microbiosensors for environmental monitoring. Sensors, Vol.8, pp.2569-2588.

Joshi, M., Kale, N., Lal, R., Ramgopal Rao, V. & Mukherji, S. 2007a. A novel dry method for surface modification of su-8 for immobilization of biomolecules in bio-mems. Biosensors and Bioelectronics, Vol.22, pp.2429-2435.

Joshi, M., Pinto, R., Rao, V. R. & Mukherji, S. 2007b. Silanization and antibody immobilization on su-8. Applied surface science, Vol.253, pp.3127-3132.

Joshi, M., Rao, V. R. & Mukherji, S. 2010. A novel technique for microfabrication of ultra-thin affinity cantilevers for characterization with an afm. Journal of Micromechanics and Microengineering, Vol.20, pp.125007.

Joshi, M., Singh, S., Swain, B., Patil, S., Dusane, R., Rao, R. & Mukherji, S. Anhydrous silanization and antibody immobilization on hotwire cvd deposited silicon oxynitride films. India Annual Conference, 2004. Proceedings of the IEEE INDICON 2004. First, 20-22 Dec. 2004 2004. pp.538-541.

Kale, N. S., Joshi, M., Nageswara Rao, P., Mukherji, S. & Ramgopal Rao, V. Bio-functionalization of silicon nitride-based piezo-resistive microcantilevers. Sadhana (Academy Proceedings in Engineering Sciences), 2009. Indian Academy of Sciences, pp.591-597.

Kandpal, M., Bandela, A. K., Hinge, V. K., Rao, V. R. & Rao, C. P. 2013. Fluorescence and piezoresistive cantilever sensing of trinitrotoluene by an upper-rim tetrabenzimidazole conjugate of calix [4] arene and delineation of the features of the complex by molecular dynamics. ACS

applied materials & interfaces, Vol.5, pp.13448-13456.

Kanungo, M., Srivastava, D. N., Kumar, A. & Contractor, A. 2002. Conductimetric immunosensor based on poly (3, 4-ethylenedioxythiophene). Chemical Communications, pp.680-681.

Ko, S. & Grant, S. A. 2006. A novel fret-based optical fiber biosensor for rapid detection of salmonella typhimurium. Biosensors and Bioelectronics, Vol.21, pp.1283-1290.

Kricka, L. & Wilding, P. 1996. Micromachining: A new direction for clinical analyzers. Pure and applied chemistry, vol.68, pp.1831-1836.

Kurihara, Y., Takama, M., Sekiya, T., Yoshihara, Y., Ooya, T. & Takeuchi, T. 2012. Fabrication of carboxylated silicon nitride sensor chips for detection of antigen–antibody reaction using microfluidic reflectometric interference spectroscopy. Langmuir, Vol.28, pp.13609-13615.

Lauks, I. R. 1998. Microfabricated biosensors and microanalytical systems for blood analysis. Accounts of chemical research, Vol.31, pp.317-324.

Lei, M., Baldi, A., Nuxoll, E., Siegel, R. A. & Ziaie, B. 2006. A hydrogel-based implantable micromachined transponder for wireless glucose measurement. Diabetes technology & therapeutics, Vol.8, pp.112-122.

Leung, A., Shankar, P. M. & Mutharasan, R. 2007. A review of fiber-optic biosensors. Sensors and Actuators B: Chemical, vol.125, pp.688-703.

Li, C. 2007. Nanobiotechnology and biosensors.

Li, P. C. H. & Harrison, D. J. 1997. Transport, manipulation, and reaction of biological cells on-chip using electrokinetic effects. Analytical chemistry, Vol.69, pp.1564-1568.

Liao, C. W., Chou, J. C., Sun, T. P., Hsiung, S. K. & Hsieh, J. H. 2007. Preliminary investigations on a glucose biosensor based on the potentiometric principle. Sensors and Actuators B: Chemical, Vol.123, pp.720-726.

Liedberg, B., Nylander, C. & Lunstrom, I. 1983. Surface plasmon resonance for gas detection and biosensing. Sensors and Actuators, Vol.4, pp.299-304.

Liu, C. X., Jiang, L. Y., Wang, H., Guo, Z. H. & Cai, X. X. 2007. A novel disposable amperometric biosensor based on trienzyme electrode for the determination of total creatine kinase. Sensors and Actuators B: Chemical, Vol.122, pp.295-300.

Madaras, M. B., Popescu, I. C., Ufer, S. & Buck, R. P. 1996. Microfabricated amperometric creatine and creatinine biosensors. Analytica chimica acta, Vol.319, pp.335-345.

Mali, P., Sarkar, A. & Lal, R. 2006. Facile fabrication of microfluidic systems using electron beam lithography. Lab on a Chip, vol.6, pp.310-315.

Marazuela, M. & Moreno-Bondi, M. 2002. Fiber-optic biosensors–an overview. Analytical and bioanalytical chemistry, Vol.372, pp.664-682.

Marie, R., Schmid, S., Johansson, A., Ejsing, L., Nordström, M., Häfliger, D., Christensen, C. B., Boisen, A. & Dufva, M. 2006. Immobilisation of DNA to polymerised su-8 photoresist. Biosensors and Bioelectronics, Vol.21, pp.1327-1332.

Melchels, F. P., Feijen, J. & Grijpma, D. W. 2010. A review on stereolithography and its applications in biomedical engineering. Biomaterials, Vol.31, pp.6121-6130.

Meyer-Plath, A., Schröder, K., Finke, B. & Ohl, A. 2003. Current trends in biomaterial surface functionalization—nitrogen-containing plasma assisted processes with enhanced selectivity. Vacuum, Vol.71, pp.391-406.

Mikkelsen, S. R. & Rechnitz, G. A. 1989. Conductometric tranducers for enzyme-based biosensors. Analytical chemistry, Vol.61, pp.1737-1742.

Myers, F. B. & Lee, L. P. 2008. Innovations in optical microfluidic technologies for point-of-care diagnostics. Lab Chip, vol.8, pp.2015-2031.

Nagy, G., Von Storp, L. & Guilbault, G. 1973. Enzyme electrode for glucose based on an iodide membrane sensor. Analytica chimica acta, Vol.66, pp.443-445.

Ni, M., Tong, W. H., Choudhury, D., Rahim, N. a. A., Iliescu, C. & Yu, H. 2009. Cell culture on mems platforms: A review. International journal of molecular sciences, Vol.10, pp.5411-5441.

Nilsson, H., Åkerlund, A. C. & Mosbach, K. 1973. Determination of glucose, urea and penicillin using enzyme-ph-electrodes. Biochimica et Biophysica

Acta (BBA)-General Subjects, Vol.320, pp.529-534.

Nordström, M., Keller, S., Lillemose, M., Johansson, A., Dohn, S., Haefliger, D., Blagoi, G., Havsteen-Jakobsen, M. & Boisen, A. 2008. Su-8 cantilevers for bio/chemical sensing; fabrication, characterisation and development of novel read-out methods. Sensors, Vol.8, pp.1595-1612.

Nuxoll, E. 2013. Biomems in drug delivery. Advanced drug delivery reviews, Vol.65, pp.1611-1625.

Ohira, S. I., Toda, K., Ikebe, S. I. & Dasgupta, P. K. 2002. Hybrid microfabricated device for field measurement of atmospheric sulfur dioxide. Analytical chemistry, Vol.74, pp.5890-5896.

Okafor, C., Grooms, D., Alocilja, E. & Bolin, S. 2008. Fabrication of a novel conductometric biosensor for detecting mycobacterium avium subsp. Paratuberculosis antibodies. Sensors, Vol.8, pp.6015-6025.

Pang, C., Zhao, Z., Du, L. & Fang, Z. 2008. Adhesive bonding with su-8 in a vacuum for capacitive pressure sensors. Sensors and Actuators A: Physical, Vol.147, pp.672-676.

Park, S. W., Kim, Y. I., Chung, K. H., Hong, S. I. & Kim, S. W. 2002. Covalent immobilization of gl-7-aca acylase on silica gel through silanization. Reactive and Functional Polymers, Vol.51, pp.79-92.

Patil, S. J., Duragkar, N. & Rao, V. R. 2014. An ultra-sensitive piezoresistive polymer nano-composite microcantilever sensor electronic nose platform for explosive vapor detection. Sensors and Actuators B: Chemical, Vol.192, pp.444-451.

Prabhakar, A. & Mukherji, S. 2010. Microfabricated polymer chip with integrated u-bend waveguides for evanescent field absorption based detection. Lab on a Chip, Vol.10, pp.748-754.

Prasad, B. & Lal, R. 1999. A capacitive immunosensor measurement system with a lock-in amplifier and potentiostatic control by software. Measurement Science and Technology, Vol.10, pp.1097.

Preejith, P., Lim, C., Kishen, A., John, M. & Asundi, A. 2003. Total protein measurement using a fiber-optic evanescent wave-based biosensor. Biotechnology letters, Vol.25, pp.105-110.

Reddy, C. V. B., Khaderbad, M. A., Gandhi, S., Kandpal, M., Patil, S., Chetty, K. N., Rajulu, K. G., Chary, P., Ravikanth, M. & Ramgopal Rao, V. 2012. Piezoresistive su-8 cantilever with fe (iii) porphyrin coating for co sensing. Nanotechnology, IEEE Transactions on, Vol.11, pp.701-706.

Rijal, K., Leung, A., Shankar, P. M. & Mutharasan, R. 2005. Detection of pathogen escherichia coli o157: H7 at 70 cells/ml using antibody-immobilized biconical tapered fiber sensors. Biosensors and Bioelectronics, Vol.21, pp.871-880.

Ruano-Lopez, J., Aguirregabiria, M., Tijero, M., Arroyo, M., Elizalde, J., Berganzo, J., Aranburu, I., Blanco, F. & Mayora, K. 2006. A new su-8 process to integrate buried waveguides and sealed microchannels for a lab-on-a-chip. Sensors and Actuators B: Chemical, Vol.114, pp.542-551.

Saliterman, S. 2006. Fundamentals of biomems and medical microdevices, SPIE press.

Schmaljohann, D. 2006. Thermo-and ph-responsive polymers in drug delivery. Advanced drug delivery reviews, Vol.58, pp.1655-1670.

Schoning, M., Jacobs, M., Muck, A., Knobbe, D. T., Wang, J., Chatrathi, M. & Spillmann, S. 2005. Amperometric pdms/glass capillary electrophoresis-based biosensor microchip for catechol and dopamine detection. Sensors and Actuators B: Chemical, Vol.108, pp.688-694.

Schöning, M., Krause, R., Block, K., Musahmeh, M., Mulchandani, A. & Wang, J. 2003. A dual amperometric/potentiometric fia-based biosensor for the distinctive detection of organophosphorus pesticides. Sensors and Actuators B: Chemical, Vol.95, pp.291-296.

Seena, V., Kale, N. S., Nag, S., Joshi, M., Mukherji, S. & Rao, V. R. 2009a. Developing a polymeric microcantilever platform technology for biosensing applications. Int. J. Micro Nano Syst, Vol.1, pp.65-70.

Seena, V., Rajorya, A., Pant, P., Mukherji, S. & Rao, V. R. 2009b. Polymer microcantilever biochemical sensors with integrated polymer composites for electrical detection. Solid State Sciences, Vol.11, pp.1606-1611.

Selvaganapathy, P., Burns, M., Burke, D. & Mastrangelo, C. Inline electrochemical detection

for capillary electrophoresis. 2001. IEEE, pp.451-454.

Shawgo, R. S., Richards Grayson, A. C., Li, Y. & Cima, M. J. 2002. Biomems for drug delivery. Current Opinion in Solid State and Materials Science, Vol.6, pp.329-334.

St. Louis, P. 2000. Status of point-of-care testing: Promise, realities, and possibilities. Clinical biochemistry, Vol.33, pp.427-440.

Staples, M., Daniel, K., Cima, M. J. & Langer, R. 2006. Application of micro-and nano-electromechanical devices to drug delivery. Pharmaceutical research, Vol.23, pp.847-863.

Studer, V., Pepin, A., Chen, Y. & Ajdari, A. 2002. Fabrication of microfluidic devices for ac electrokinetic fluid pumping. Microelectronic Engineering, Vol.61, pp.915-920.

Suter, M., Li, Y., Sotiriou, G., Teleki, A., Pratsinis, S. & Hierold, C. Low-cost fabrication of pmma and pmma based magnetic composite cantilevers. Solid-State Sensors, Actuators and Microsystems Conference (TRANSDUCERS), 2011 16th International, 2011. IEEE, pp.398-401.

Svasek, P., Svasek, E., Lendl, B. & Vellekoop, M. 2004. Fabrication of miniaturized fluidic devices using su-8 based lithography and low temperature wafer bonding. Sensors and Actuators A: Physical, Vol.115, pp.591-599.

Tamayo, J., Kosaka, P. M., Ruz, J. J., San Paulo, Á. & Calleja, M. 2013. Biosensors based on nanomechanical systems. Chemical Society Reviews, Vol.42, pp.1287-1311.

Tang, L., Ren, Y., Hong, B. & Kang, K. A. 2006. Fluorophore-mediated, fiber-optic, multi-analyte, immunosensing system for rapid diagnosis and prognosis of cardiovascular diseases. Journal of Biomedical Optics, Vol.11, pp.021011.

Tao, S. L., Popat, K. C., Norman, J. J. & Desai, T. A. 2008. Surface modification of su-8 for enhanced biofunctionality and nonfouling properties. Langmuir, Vol.24, pp.2631-2636.

Terry, S. C., Jerman, J. H. & Angell, J. B. 1979. A gas chromatographic air analyzer fabricated on a silicon wafer. IEEE Transactions on Electron Devices, Vol.26, pp.1880-1886.

Thaysen, J., Marie, R. & Boisen, A. Cantilever-based bio-chemical sensor integrated in a microliquid handling system. Micro Electro Mechanical Systems, 2001. MEMS 2001. The 14th IEEE International Conference on, 2001. IEEE, pp.401-404.

Tromberg, B. J., Sepaniak, M. J., Vo-Dinh, T. & Griffin, G. D. 1987. Fiber-optic chemical sensors for competitive binding fluoroimmunoassay. Analytical chemistry, Vol.59, pp.1226-1230.

Tschmelak, J., Proll, G. & Gauglitz, G. 2004. Sub-nanogram per litre detection of the emerging contaminant progesterone with a fully automated immunosensor based on evanescent field techniques. Analytica chimica acta, Vol.519, pp.143-146.

Türkarslan, Ö., Kayahan, S. K. I. & Toppare, L. 2009. A new amperometric cholesterol biosensor based on poly (3, 4-ethylenedioxypyrrole). Sensors and Actuators B: Chemical, Vol.136, pp.484-488.

Van Den Berg, A., Grisel, A., Verney-Norberg, E., Van Der Schoot, B. H., Koudelka-Hep, M. & De Rooij, N. F. 1993. On-wafer fabricated free-chlorine sensor with ppb detection limit for drinking-water monitoring. Sensors and Actuators B: Chemical, Vol.13, pp.396-399.

Wang, H., Meng, S., Guo, K., Liu, Y., Yang, P., Zhong, W. & Liu, B. 2008. Microfluidic immunosensor based on stable antibody-patterned surface in pmma microchip. Electrochemistry Communications, Vol.10, pp.447-450.

Wang, J., Pumera, M., Chatrathi, M. P., Escarpa, A., Musameh, M., Collins, G., Mulchandani, A., Lin, Y. & Olsen, K. 2002a. Single-channel microchip for fast screening and detailed identification of nitroaromatic explosives or organophosphate nerve agents. Analytical chemistry, vol.74, pp.1187-1191.

Wang, J., Pumera, M., Collins, G. E. & Mulchandani, A. 2002b. Measurements of chemical warfare agent degradation products using an electrophoresis microchip with contactless conductivity detector. Analytical chemistry, Vol.74, pp.6121-6125.

Wang, X., Dzyadevych, S. V., Chovelon, J.-M., Renault, N. J., Chen, L., Xia, S. & Zhao, J. 2006. Development of a conductometric nitrate biosensor based on methyl viologen/nafion® composite film. Electrochemistry communications, Vol.8, pp.201-205.

Wang, Y., Pai, J.-H., Lai, H.-H., Sims, C. E., Bachman, M., Li, G. & Allbritton, N. L. 2007. Surface graft polymerization of su-8 for bio-mems applications. Journal of Micromechanics and Microengineering, Vol.17, pp.1371.

Wang, Y., Vaidya, B., Farquar, H. D., Stryjewski, W., Hammer, R. P., Mccarley, R. L., Soper, S. A., Cheng, Y.-W. & Barany, F. 2003. Microarrays assembled in microfluidic chips fabricated from poly (methyl methacrylate) for the detection of low-abundant DNA mutations. Analytical Chemistry, Vol.75, pp.1130-1140.

Waswa, J., Irudayaraj, J. & Debroy, C. 2007. Direct detection of e. Coli o157: H7 in selected food systems by a surface plasmon resonance biosensor. LWT-Food Science and Technology, Vol.40, pp.187-192.

Watson, L., Maynard, P., Cullen, D., Sethi, R., Brettle, J. & Lowe, C. 1988. A microelectronic conductimetric biosensor. Biosensors, Vol.3, pp.101-115.

Wicker, R. B., Medina, F. & Elkins, C. Multiple material micro-fabrication: Extending stereolithography to tissue engineering and other novel applications. Proceedings of 15th Annual Solid Freeform Fabrication Symposium, Austin, TX, 2004. Wiley, pp.754-64.

Xia, Y. & Whitesides, G. M. 1998. Soft lithography. Annual review of materials science, Vol.28, pp.153-184.

Yang, Z. 2003. Implantable biosensor. Google Patents.

Ymeti, A., Greve, J., Lambeck, P. V., Wink, T., Van Hövell, S. W. F. M., Beumer, T. a. M., Wijn, R. R., Heideman, R. G., Subramaniam, V. & Kanger, J. S. 2007. Fast, ultrasensitive virus detection using a young interferometer sensor. Nano letters, Vol.7, pp.394-397.

Yoon, S.-I., Lim, M.-H., Park, S.-C., Shin, J.-S. & Kim, Y.-J. 2008. Neisseria meningitidis detection based on a microcalorimetric biosensor with a split-flow microchannel. Microelectromechanical Systems, Journal of, Vol.17, pp.590-598.

Yu, H. & Shiquan, T. 2011. An optical fiber sensor probe using a pmma/cpr coated bent optical fiber as a transducer for monitoring trace ammonia. Journal of Sensor Technology, vol.2011.

Zhao, X.-M., Xia, Y. & Whitesides, G. M. 1997. Soft lithographic methods for nano-fabrication. J. Mater. Chem., Vol.7, pp.1069-1074.

Ziaie, B., Baldi, A., Lei, M., Gu, Y. & Siegel, R. A. 2004. Hard and soft micromachining for biomems: Review of techniques and examples of applications in microfluidics and drug delivery. Advanced Drug Delivery Reviews, Vol.56, pp.145-172.

V.Ramgopal Rao is an Institute Chair Professor at Indian Institute of Technology Bombay at the Department of Electrical Engineering. He received the prestigious Infosys Science Foundation Prize 2013 in Engineering and Computer Science among the numerous national and international awards. His contributions range from integrating chemistry with mechanics and electronics for the invention of new devices of societal impact, nanoscale electronics and innovation and entrepreneurship. He has more than 300 papers in leading international journals and prestigious international conferences and 16 patents issued or pending.

Gaurav Chatterjee received his Bachelors of Engineering in Electronics and Telecommunications Engineering from University of Mumbai in 2007 and Master of Science (Thesis) in Electrical Engineering from Arizona State University in 2009. He is currently pursuing his Ph.D as a Erasmus Mundus Fellow from LIRMM, France. He worked as a Senior Researcher at Indian Institute of Technology Bombay and worked for the development of low cost point of care diagnostics. He has been actively involved in the developing of MEMS based biosensors. His research interests include Bio-MEMS and microfluidics and MEMS sensors for space applications.

Nidhi Maheshwari received her Ph.D. in Biosciences and Bioengineering from Indian Institute of Technology Bombay in 2012 and Master of Science in Biotechnology. She is currently working as Project Manager for MEMS and NEMS Prototyping Facility, at IIT Bombay funded by the Department of Electronics and Information Technology, Government of India. She has been actively involved in the development of low cost point of care diagnostics. Her research interests include MEMS based biosensors and microfluidics for bio-medical applications, polymer based sensors and developing low cost processes for polymer microfabrication.