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BioMEMS: state-of-the-art in detection, opportunities and prospects

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Abstract

In recent years, the biological and biomedical applications of micro- and nanotechnology (commonly referred to as Biomedical or Biological Micro-Electro-Mechanical Systems [BioMEMS]) have become increasingly prevalent and have found widespread use in a wide variety of applications such as diagnostics, therapeutics, and tissue engineering. While research and development activity in this field stays intense, some applications have also been commercialized. This article reviews the recent advances in this very exciting and important field and presents a summary of the state of the art in the area of BioMEMS focusing on diagnostics, sensing, and detection. The areas of therapeutics and hybrid bio/artificial devices will be presented in more detail elsewhere [Biomedical Nanotechnology, Vol. I–IV, Maruo Ferrari (Ed.), Kluwer Academic Publishers, 2004, in press.] and here are discussed briefly in terms of future directions and prospects.

Keywords: BioMEMS; Biochips; Lab-on-chip; Nanotechnology; Nanobiotechnology

Contents

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1. Introduction and BioMEMS defined

Since the inception of micro-electro-mechanical systems in the early 1970s, the significance of the biomedical applications of these miniature systems were realized [1,2]. Biomedical or Biological Micro-Electro-Mechanical Systems (BioMEMS) are now a heavily researched area with a wide variety of important biomedical applications [3]. In general, BioMEMS can be defined as "devices or systems, constructed using techniques inspired from micro/nano-scale fabrication, that are used for processing, delivery, manipulation, analysis, or construction of biological and chemical entities". These devices and systems encompass all interfaces of the life sciences and biomedical disciplines with micro- and nano-scale systems. Areas of research and applications in BioMEMS range from diagnostics, such as DNA and protein micro-arrays, to novel materials for BioMEMS, micro-fluidics (not dealt with in this review), tissue engineering, surface modification, implantable BioMEMS, systems for drug delivery, etc. A large number of MEMS for biology and medicine have been presented (reviewed in Refs. [4–7]). The devices and integrated systems using BioMEMS are also known as lab-on-a-chip and micro-total analysis systems (micro-TAS or μTAS). The word is now used very broadly and devices which do not have any electro-mechanical components, such as DNA and protein arrays (described briefly in the following sections), are also sometimes categorized under BioMEMS. Fig. 1 shows a schematic drawing of the key segments of research areas resulting from integration of life sciences and biomedical disciplines with micro- and nano-scale systems. The areas on the right are applications of biology to micro- and nano-scale systems and materials, while the areas on the left are applications of micro- and nano-scale systems to biological and biomedical problems.

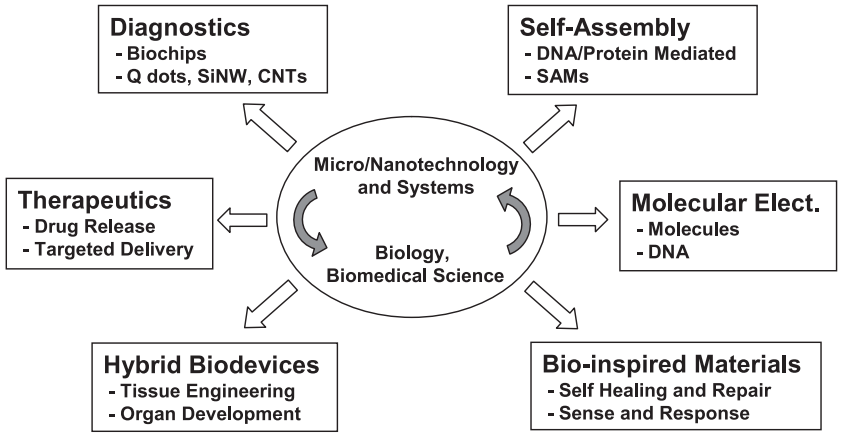

Fig. 1. Research areas resulting from the integration of micro- and nano-scale systems and biomedical sciences.

2. Materials used

BioMEMS and related devices can be fabricated with three classes of materials. These can be categorized as (i) microelectronics related materials, such as silicon, glass, and related materials used for microelectronics and MEMS, (ii) plastic and polymeric materials such (poly)dimethylsiloxane) (PDMS), etc., and (iii) biological materials and entities such as proteins, cells, and tissues. The first class of materials has been reported on extensively, both from a research and implementation point of view, and has traditionally been used in MEMS and devices [2,4,5]. Processing of BioMEMS devices using polymer devices and soft lithography is very attractive due to increased biocompatibility and ease in fabrication [8], ability to integrate functional hydrogel materials [9], and low cost and rapid prototyping methods available in plastic materials [10,11]. The use of these materials for practical applications continues to increase steadily. The work encompassing the third class of materials is relatively unexplored, represents many new and exciting possibilities, and will form the new frontier of BioMEMS and bionanotechnology, for example, in the application of micro- and nanotechnology-inspired cell and tissue engineering and in developing the tools for understanding cellular functions and systems biology. The use of micro- and nano-fabrication techniques for the 'directed' synthesis and construction of biological structures, such as artificial organs and hybrid devices, presents a wide spectrum of opportunities for research and applications [12]. Applications such as development of cell-based arrays, micro-fabrication-mediated tissue engineering [13], and development of artificial organs using micro- and macro-scale construction techniques [14] are some of the many very exciting possibilities in the horizon (and will be discussed more in Section 5).

3. BioMEMS for diagnostic applications

Diagnostics represents the largest and most researched BioMEMS segment. A very large and increasing numbers of BioMEMS devices for diagnostic applications have been developed and presented in the literature by many groups within the last few years. These devices differ significantly in their designs and fabrication techniques and also in the areas of their applications. BioMEMS for diagnostic applications are also sometimes referred to as 'BioChips'. These devices are used to detect cells, microorganisms, viruses, proteins, DNA and related nucleic acids, and small molecules of biochemical importance and interest. In general, the use of micro- and nano-scale detection technologies is justified by (i) reducing the sensor element to the scale of the target species and hence providing a higher sensitivity, (ii) reduced reagent volumes and associated costs, (iii) reduced time to result due to small volumes resulting in higher effective concentrations, and (iv) amenability of portability and miniaturization of the entire system. We will introduce some select examples of BioMEMS for diagnostic applications below. Firstly, BioMEMS detection modalities are presented, followed by some examples of BioMEMS and biochips sensors. Then DNA micro-arrays, protein micro-arrays, and lab-on-a-chip using micro-fluidics are briefly reviewed. The DNA and protein micro-arrays could be very powerful BioMEMS platforms for rapid detection, drug discovery, and screening, especially when combined with integrated micro-fluidics and sensitive detection technologies.

3.1. Detection methods, BioMEMS, and biochip sensors

Biosensors are analytical devices that combine a biologically sensitive element with a physical or chemical transducer to selectively and quantitatively detect the presence of specific compounds in a given external environment [15]. During the last decade, BioMEMS and devices have been used as biosensors and the resulting biochips can allow sensitive, rapid, and real-time measurements [16,17]. These BioMEMS sensors can be used to detect cells, proteins, DNA, or small molecules. Many demonstrations to date are on one sensor and these sensors can potentially be integrated into an array format. There are many detection methods used in BioMEMS sensors and biochips, including (i) mechanical, (ii) electrical, (iii) optical, etc. Fig. 2 shows a schematic of these key detection modalities as they are used in biochips and BioMEMS sensors.

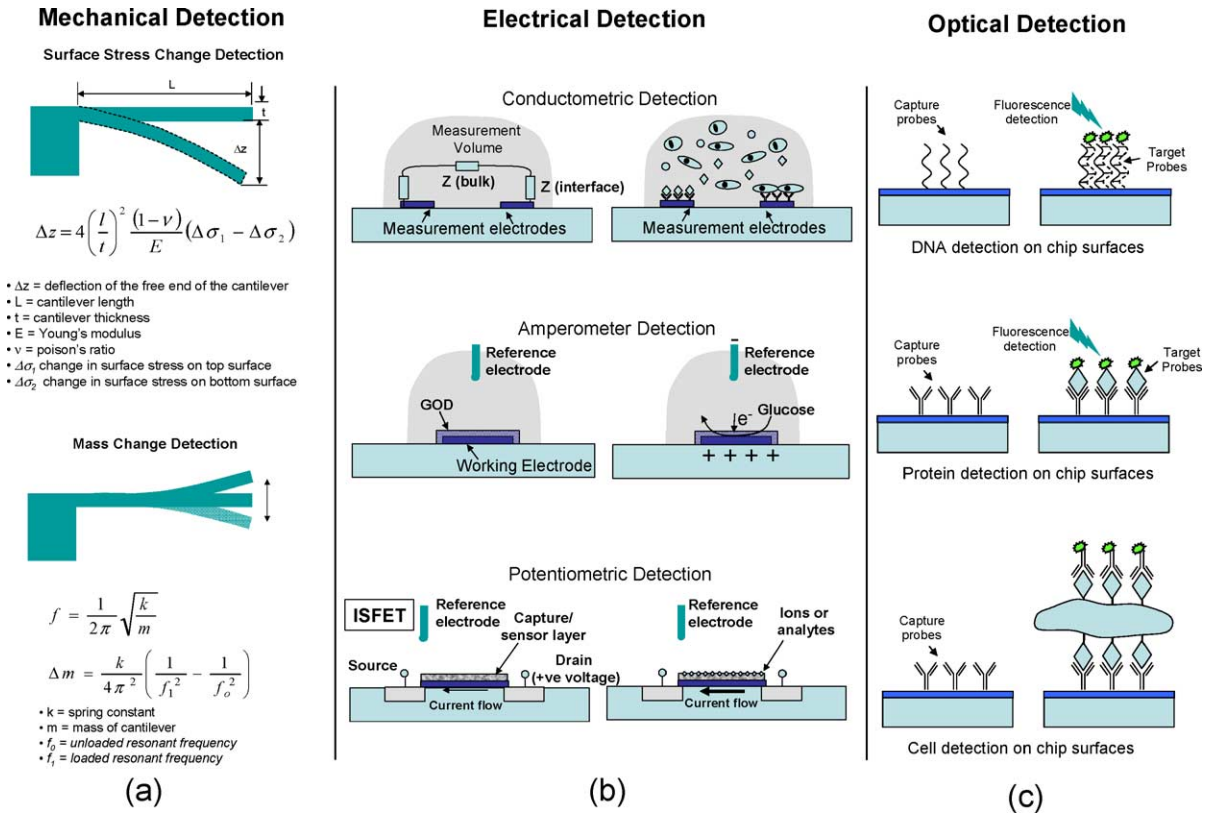

Fig. 2. Key detection modalities used in BioMEMS and biochip sensors.

3.1.1. BioMEMS and mechanical detection

Mechanical detection for biochemical entities and reactions has more recently been used through the use of micro- and nano-scale cantilever sensors on a chip. As shown in Fig. 2(a), these cantilever sensors (diving board type structures) can be used in two modes, namely stress sensing and mass sensing. In stress sensing mode, the biochemical reaction is performed selectively on one side of the cantilever. A change in surface free energy results in a change in surface stress, which results in measurable bending of the cantilever. Thus, label-free detection of biomolecular binding can be performed. The bending of the cantilever can then be measured using optical means (laser reflecting from the cantilever surface into a quad position detector, like in an AFM) or electrical means (piezo-resistor incorporated at the fixed edge of the cantilever). To increase the stress sensitivity of the cantilever, the spring constant should be reduced, while the overall surface of the cantilever determines the number of molecules that should attach to the surface to cause a resulting stress change. In the mass sensing mode, the cantilever is excited mechanically so that it vibrates at its resonant frequency (using external drive or the ambient noise, for example). The resonant frequency is measured using electrical or optical means, and compared to the resonant frequency of the cantilever once a biological entity is captured. The change in mass can be detected by detection of shift in resonant frequency, assuming the spring constant does not change. To increase the mass sensitivity, in general, the mass of the cantilever should be made smaller, the quality factor should be increased, the resonant frequencies should be designed such that it is easily measured, and the detection system should be designed to measure as small of frequency shift as possible. The quality factor is decreased with increased damping, for example, in a fluid, and hence the minimum detectable mass is much higher in damped mediums as compared to low-

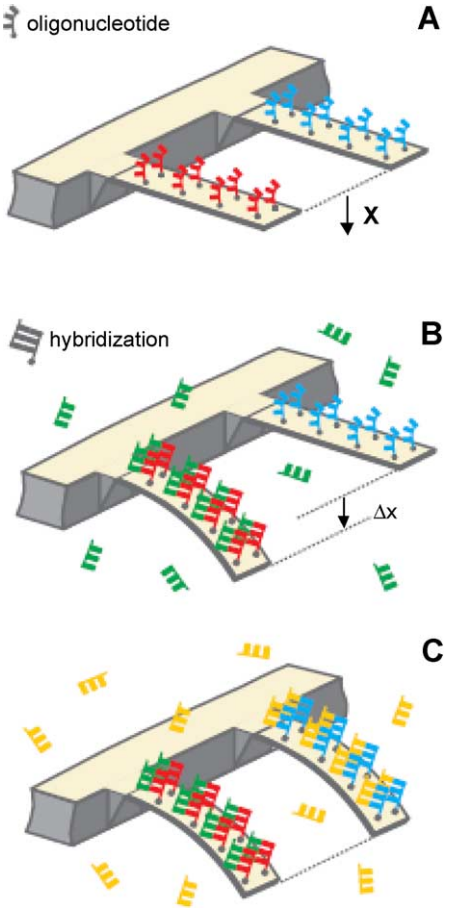

Fig. 3. Detection of label-free DNA hybridization using micromechanical cantilevers. Reprinted with permission from Science 288 (2000) 316–318 AAAS and with kind permission from J.K. Gimzewski.

damped mediums. Thus, the stress detection mode is inherently preferred in a fluid.

One of the main advantages of the cantilever sensors is the ability to detect interacting compounds without the need of introducing an optically detectable label on the binding entities. In the recent years, very exciting and significant advances in biochemical detection have been made using cantilever sensors. Direct, label-free detection of DNA and proteins have been demonstrated (schematically shown in Fig. 3) using silicon cantilevers [18]. Hybridization of DNA and detection of single based mismatches on DNA strands has been demonstrated on cantilevers with a thin Au gold layer on one side [19–21]. Thiolated capture DNA strands are attached to the Au layer and the deflection of cantilevers can be detected when the target strands bind to the capture strands. These sensors can also be used to detect proteins and cancer markers such prostate specific antigen, which have also been detected at 0.2 ng/ml in background of human serum albumen in clinically relevant conditions, as shown in Fig. 4 [22]. Cantilever arrays have also been demonstrated to measure analyte vapors in the gas phase by change in surface stress, as an artificial nose [23]. Cantilevers coated with environmentally sensitive hydrogels such as pH-sensitive (poly)methacrylic acid (PMAA) can also be used to induce a stress on the cantilever surface since these polymers are known to expand (or contract) upon change in pH. Highly sensitive pH detectors,

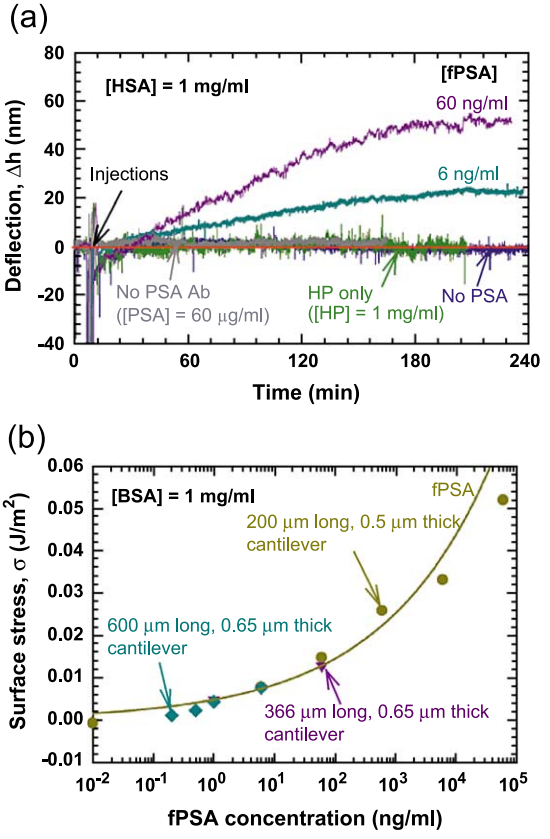

Fig. 4. Detection of prostate specific antigen using microcantilevers in clinically relevant conditions, showing surface stress as a geometry-independent parameter for assaying PSA Yu et al. [42]. Reprinted with permission from Nat. Biotechnol. 19 (2001) 856–860 and with kind permission from A. Majumdar.

capable of detecting a change in pH of 1e − 4 to 1e − 5 within a pH range of 5–6 have also been demonstrated [24,25].

The capture of larger entities such as cells on antibodies attached to cantilevers has not been reported using the stress detection method. Since the stress detection method used with cantilevers is based upon a change in surface energy, it can be speculated that the DNA or protein layers are continuous over the area of gold-coated cantilevers, as is the case with Self-Assembled Monolayers (SAMs), and hence result in a uniform surface stress change, resulting in the cantilever bending. The capture of larger entities such as cells on antibodies attached to a cantilever might not produce such stress changes. However, detection of cells and microorganisms has been demonstrated using mass detection method employing a shift in resonant frequency. Various examples of mass demonstrations are reported in literature, for example, detection of the mass of *Escherichia coli* O157:H7 was detected using cantilevers [26,27], detection of mass of single vaccinia virus particle, as shown in Fig. 5 [28], and mass change in a polymer upon absorption of vapor [29].

3.1.2. BioMEMS and electrical detection

Electrical or electrochemical detection techniques have also been used quite commonly in biochips and BioMEMS sensors. These techniques can be amenable to portability and miniaturization, when compared to optical detection techniques, however, recent advances in integration optical components on a chip can also produce smaller integrated devices [30,31]. Electrochemical biosensors include three basic types, as shown in Fig. 2(b), they are as follows: (i) amperometric biosensors, which involves the electric current associated with the electrons involved in redox processes, (ii) potentiometric biosensors, which measure a change in potential at electrodes due to ions or chemical reactions at an electrode (such as an ion Sensitive FET), and (iii) conductometric biosensors, which measure conductance changes associated with changes in the overall ionic medium between the two electrodes. There are more reports on potentiometric and amperometric sensors, specially, due to the established field of electrochemistry, and many of these sensors have been used as the micro- and nano-scale.

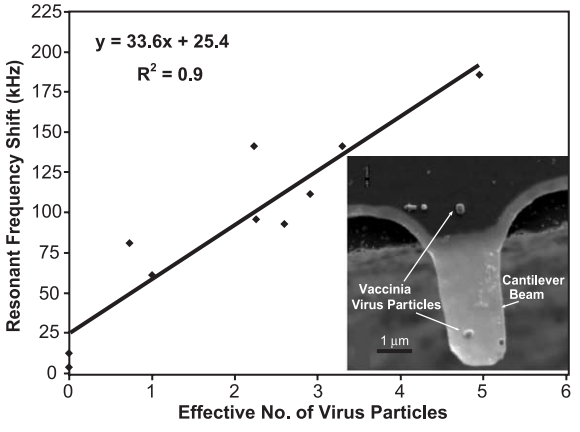

Fig. 5. Shift (decrease) in resonant frequency with increasing number of virus particles. Inset shows an SEM of a nano-cantilever with a single Vaccinia virus particle Gupta et al. [28]. Reprinted with permission from Appl. Phys. Lett. 84 (10) (2004) and with kind permission from R. Bashir.

The most prevalent examples of amperometric biosensors employ an enzyme-catalyzed redox reaction, where the resulting redox electron current is measured at a working electrode. The most widely used examples are that of detection of glucose, based on glucose oxidase, which generates hydrogen peroxide and gluconic acid in the presence of oxygen, glucose, and water [32]. Then, hydrogen peroxide is reduced at − 600 mV at Ag/AgCl anode reference electrode. These devices are designed either for monitoring formation of hydrogen peroxide formation or consumption of oxygen. At the micro-scale, these sensors require the formation of the working and reference electrodes on a chip, and an enzymatic layer on the working electrode, as demonstrated for the detection of glucose, lactose, and urea [33,34] and for the detection of glucose [35]. More recently, hydrogels and conducting electroactive polymers have been integrated to develop electroactive hydrogels that physically entrap enzymes within their matrices for biosensor construction and chemically stimulated controlled release. Using these materials, the fabrication of glucose, cholesterol, and galactose amperometric biosensors has been demonstrated on a chip [36,37]. In addition, amperometric biosensors on a chip have been applied towards detection of gases [38], metabolic parameters in human blood [39], lactate [40], and even DNA hybridization [41]. The detection of DNA hybridization, performed by site-specific incorporation of ferrocenyl derivatives into DNA oligonu-

cleotides that function as electrochemical probes [41], is also being commercialized [42,43]. The ferrocene-modified DNA oligonucleotides prepared from phosphoramidites I and II (E1/2 of 0.120 V vs. Ag/AgCl) act as signaling probes for the electronic detection of nucleic acids using DNA chips. A full CMOS chip with a specialized backend process has also been developed for the detection of DNA using a redox-cycling based electrochemical technique [44].

Potentiometric sensors utilize the measurement of a potential at an electrode in reference to another electrode. The most common form of potentiometric sensors are the ion-sensitive field effect transistors (ISFETs) or chemical field effect transistors (ChemFETs). These devices are available commercially as pH sensors and many examples have been reported in literature [45]. Potentiometric sensors with ion-selective ionophores in modified poly(vinyl chloride) (PVC) has been used to detect analytes from human serum [34]. Cellular respiration and acidification due to the activity of the cells has been measured with CMOS ISFETS [46]. Light-addressable potentiometric sensor (LAPS) have been used to detect the change in hydrogen ion concentration and hence the pH using a field effect device in silicon in presence of light [47,48]. Potentiometric sensors have been down-scaled to nano-meter dimension through the use of silicon nano-wires, as schematically shown in Fig. 6, [49] and carbon nanotubes as field effect sensors [50], to take advantage of enhance sensitivity due to higher surface area to volume ratio. The integration of these nano-scale sensors in lab-on-chips is more challenging but recent advances in top-down fabrication techniques have been use to demonstrate such nano-scale structures [51,52], as depicted in Fig. 7 (adapted from Ref. [52]). Potentiometric sensors at the micro-scale have also been used to perform label-free detection of hybridization of DNA [53]. These sensors were incorporated within cantilevers so that they can be used within micro-fluidic channels. The DNA hybridization was detected by measuring the field effect in silicon by the intrinsic molecular charge on the DNA, using a buffer of poly-L-lysine later.

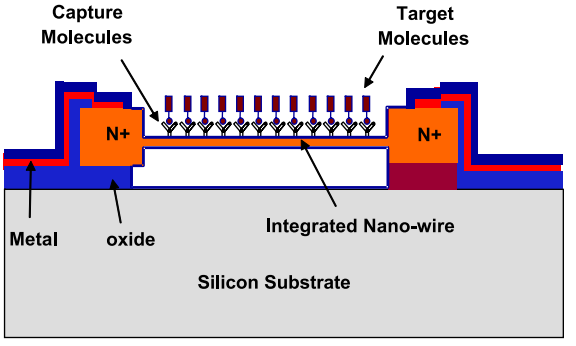

Fig. 7. A schematic of an integrated nano-wire sensor (adapted from Elibol et al. [52]).

Conductometric sensors measure the changes in the electrical impedance between two electrodes, where the changes can be at an interface or in the

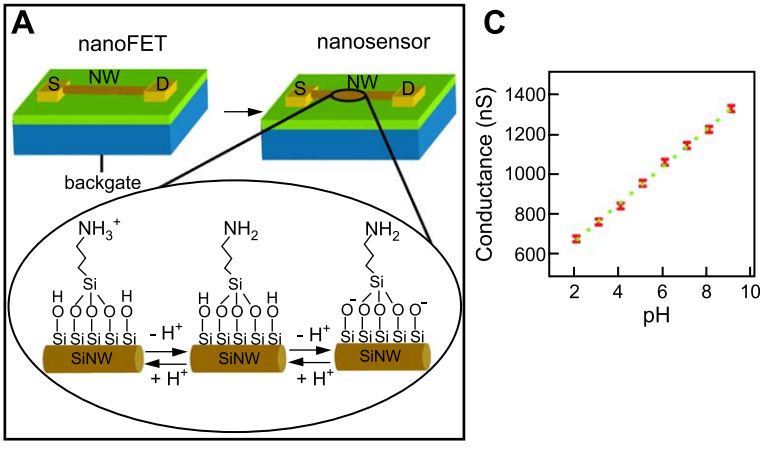

Fig. 6. A nano-wire potentiometric sensor for pH detection Cui et al.[49,50]. Reprinted with permission from Science 293 (August 17, ©2001) 1289–1292 AAAS and with kind permission from C. Leiber.

bulk region and can be used to indicate biomolecular reaction between DNA, proteins, and antigen/antibody reaction, or excretion of cellular metabolic products. Micro-fabricated devices have been used to measure extracellular neuronal activity for a long time [54,55] (the entire area of neuro-electric interface needs a review in itself). Conductance techniques are attractive due to their simplicity and ease of use since a specialized reference electrode is not needed, and have been used to detect a wide variety of entities such as agents of biothreat [56], biochemicals [57], toxins [58], and nucleic acids [59,60]. Conductometric sensors provide information on the ionic strength in electrolytes and can provide selectivity if coupled with enzyme membranes. These sensors have been used to detect different analytes, for example, urea, glucose, etc. [61,62]. Measurement of impedance (or admittance) was used to measure the metabolic activity of microorganisms within micro-fluidic biochips. As bacterial cells are grown within micro-fluidic channels and wells, the impedance changes in the medium can be detected using electrodes placed appropriately within the channels [63]. Electrical measurements of DNA hybridization using conductance techniques have been demonstrated where the binding of oligonucleotides functionalized with gold nanoparticles leads to conductivity changes associated with binding events [64]. A subsequent silver deposition on the gold nano-particles can be used to readily measurable conductivity changes, and this approach is also being commercialized [65].

Cell-based sensors are also an important class of sensors, gaining more attention in recent years. The use of cells as sensors is a very attractive way to devise sensitive biochemical detectors, as shown schematically in Fig. 8. With their highly selective and sensitive receptors, channels, and enzymes, intact cells are very attractive candidates for the development of biosensors. The main advantages of the cells as biosensors are that cells have built-in natural selectivity to biologically active chemicals and they can react to analytes in a physiologically relevant mode [66–68]. The transductions of the cell sensor signals maybe achieved by the measurement of transmembrane and cellular potentials, impedance changes, metabolic activity, analyte inducible emission of genetically engineered reporter signals, and optically by means of fluorescence or luminescence.

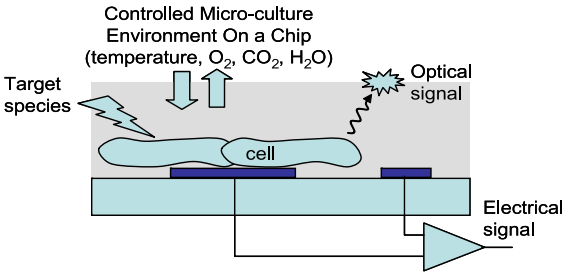

Fig. 8. Schematic of a cell-based sensor. The device can also be in an array format where many cells or single cells are interrogated upon external stimulus.

Neurons have been cultured on micro-fabricated surfaces and changes in their electrical signals upon exposure to harmful chemicals and toxins have been measured on a chip [55,69]. Chick cardiac myocytes were cultured on platinized gold electrodes to measure the electrical activity of the cells and their use in cell-based biosensor [70]. Significant challenges exist for long-term operation since the cells need to be kept alive and healthy under various harsh operating conditions and much work has been done towards this front, as this technology has been extended to demonstrate automated portable cell based biosensors platform that have been field tested [70,71] (same issue pp. 543–577). Genetically engineered B cells have been used as sensors, which emit light once they have been infected by a toxin or a virus [72]. Liver cells have also been used as biosensors by culturing them in 3-D culture environment for over 14 days and the toxicity of the target compounds was determined optically [73,74]. Microorganisms have also been used as biosensors for the detection and monitoring of environmental pollutants [75,76]. Direct measurement of current through ion channels in the cells has also been used to develop on-chip patch clamp devices [77,78], which can potentially be very sensitive to changes in the ambient conditions of the cells [79,80]. Such signal cell measurements can be very useful for drug discovery [81], biosensors, and understanding the biochemical signaling pathways of cells for systems biology applications (see later section). Whole cell-based sensors will potentially offer tremendous benefits for the evaluation of drug candidates and effects of biochemicals on multi-cellular organisms since the response of these sensors is directly predictive of the physiological response of an organism.

3.1.3. BioMEMS and optical detection

Optical detection techniques are perhaps the most common due to their prevalent use in biology and life sciences. There is a very significant amount of literature on BioMEMS devices with optical detection. A brief overview is presented here. Optical detection techniques can be based on fluorescence or chemiluminescence. Fluorescence detection techniques are based on fluorescent markers that emit light at specific wavelengths and the presence and enhancement, or reduction (as in Fluorescence Resonance Energy Transfer) in optical signal can indicate a binding reaction, as shown schematically in Fig. 2(c). The additional requirement of attachment of the capture entities on the surface of the chips, which can be metal like gold, or insulators such as silicon dioxide, needs to be carefully considered. Proper attachment of DNA [82–84], proteins [85–88], and other molecules is very critical to efficient capture of the target species. Recent advances in fluorescence detection technology have enabled single molecule detection [15,89,90]. Fluorescence-based detection in BioMEMS has been applied to detection of cells within micro-chips, using antibody-based (ELISA type) assays as shown in Fig. 9 [90,91]. Majority of the detection schemes in micro-array and numerous lab-on-a-chip devices and applications (as described in the next section) utilize optical detection schemes. Detection of proteins [92] and detection of DNA using PCR on a chip [93] are among a few examples. Within photo-definable hydrogel-based micro-chambers of a micro-fluidic chip, single-stranded DNA was immobilized on mi-

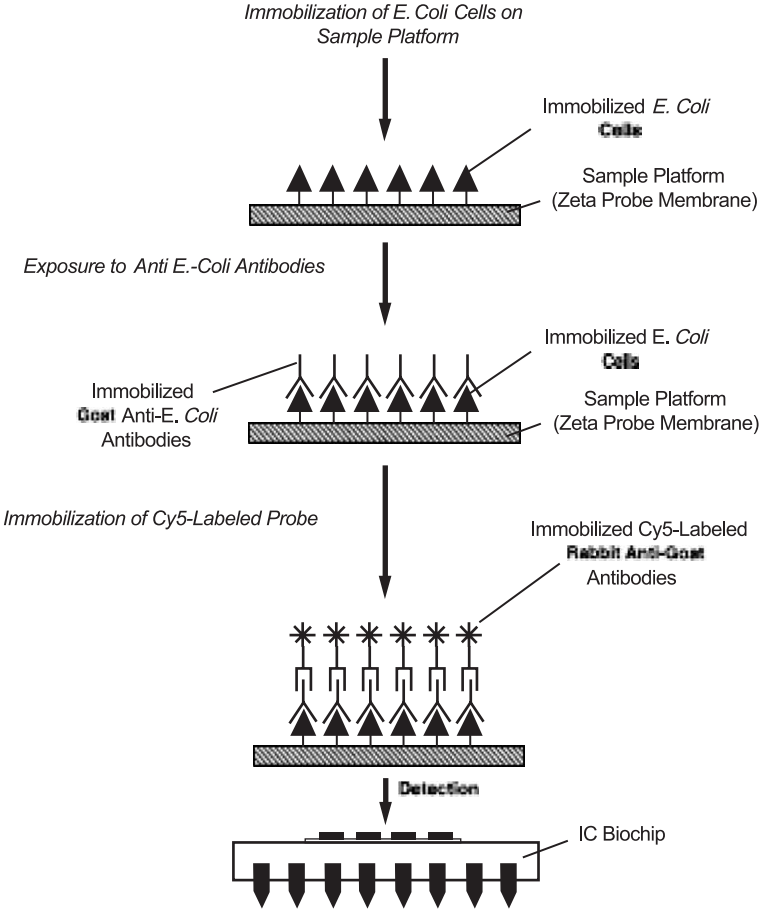

Fig. 9. Optical detection of *E. coli* using fluorescently labeled antibodies on a chip [15]. Reprinted with permission from Fresenius' J. Anal. Chem. 369 (©2001) 295 and with kind permission from T. Vo-Dinh.

cro-beads and the beads were trapped in these micro-chambers after which the complementary strands of fluorescently labeled DNA were injected and successfully hybridized within 1 min [94]. This type of technique was also able to discriminate single-nucleotide mismatches at femtomolar DNA concentrations [95,96].

Chemiluminescence is the generation of light by the release of energy as a result of a chemical reaction. Chemical reactions using synthetic compounds and usually involving a highly oxidized species, such as a peroxide, are commonly termed chemiluminescent reactions. Light emission from a living organism is commonly termed bioluminescence (sometimes called biological fluorescence), and light emission which take place by passage of electrical current is designated electrochemiluminescence. Prototype biochips for point-of-care diagnostics based on bioluminescence have been reported [97]. Bioluminescent light generated from a 1-mM ATP with firefly luciferase/luciferin solution was placed inside the channels and chambers, coated with metal, and the light output was observed through a close up lens by a CCD, with maximum light enhancement obtained by silver coated microchannels and chambers. Similar enhancements in optical sensitivity can be achieved when chemiluminescence is combined with three-dimensional channels in biochips for quantitative detection of hybridization [98] and for capillary electrophoresis in PDMS [99]. One of the challenges for optical detection within biochips is the ability to integrate the detectors in a miniaturized portable format. This integration requires fabrication of photo-diodes in silicon substrates [100] or heterogeneous integration of compound semiconductor LEDs and photodetectors within plastic or polymer platforms [31]. In the later study, microassembly of a hybrid fluorescence detection microsystem was demonstrated by heterogeneous integration of a CdS thin-film filter, an (In,Ga)N thin-film blue LED, and a disposable PDMS micro-fluidic device onto a Si PIN photodetector substrate. Miniaturization of electrophoresis devices, biomolecular sensors, and detectors has been of wide interest and as the quantity of reagents, sample, and labels are reduced, the demands on improving signal to noise ratio and sensitivity are increased [101,102].

3.2. Micro-array technology

It should be noted that any of the sensors described above can be developed into an array format to detect multiple entities simultaneously. However, DNA micro-arrays have become the most successful example of the merger between microelectronics technologies, biology, and chemistry. The techniques used to define patterns on semiconductor surfaces were utilized to construct arrays of single-stranded DNA. Once single strands of known sequences (capture probes) are placed at specific known sites on a chip surface, hybridization with molecules of unknown sequence (target probes) can reveal the sequence. There are two basic approaches to 'forming' the DNA arrays, namely optical and electrical. The optical approach, shown in Fig. 10, uses a mask to selectively de-protect sites where chemical reactions can be performed to build the molecule, one base at a time [103]. The DNA

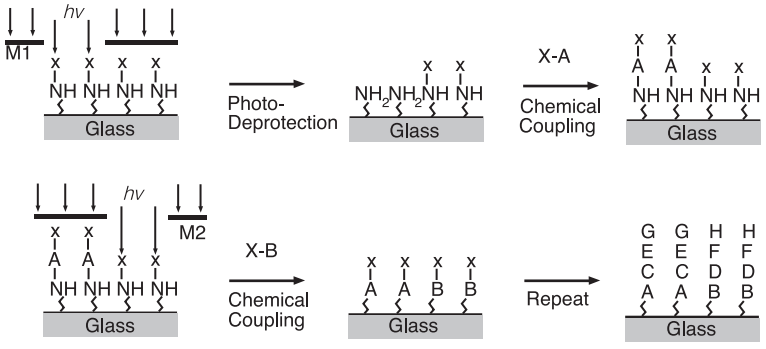

Fig. 10. Light-directed synthesis of DNA micro-arrays using spatially addressable parallel chemical synthesis. Reprinted with permission from Science 251 (February 15, ©1991) 767 AAAS and with kind permission from S.P.A. Fodor.

arrays prepared using this technique requires a large number of masking steps, but this approach can potentially lead to a higher density of molecules with a certain number of masking steps. The other approach takes advantage of the fact that oligonucleotides and DNA have a negative charge, due to the phosphate back-bone, as shown in Fig. 2, and can be electrophoretically transported to specified locations on chip surfaces [104]. This can also result in higher local concentration and accelerated DNA hybridization and electronic stringency [105–108]. The electrical approach can be used to address each pixel with the entire molecule and the array can be built pixel by pixel, by the user, as shown in Fig. 11. Both the above approaches are now being commercialized for single nucleotide polymorphisms (SNPs), short tandem repeats (STRs), insertions, deletions, and other genetic mutations [109,110].

The detection of hybridization, in both cases, is typically done by optical means (fluorescence) but can also be done electrically [42,43,111]. Electrical detection of DNA hybridization is a very sought after goal, since the possible goal of performing 'label-free' detection of DNA or protein binding can result in ease of use, reduced reagents and processing costs, and amenability to portability and miniaturization. Cantilever sensors, as described above, have been used to detect DNA hybridization without the use of any labels.

Protein and antibody arrays can play a key role in search for disease-specific proteins that have medical, diagnostic, prognostic, and commercial potential as disease markers or as drug targets and for determination of predisposition to specific disease via genotypic screening (reviewed in detail in Refs. [35,112–114]). With the recent advancements in genomics and proteomics technologies, such as sequencing robotics, mass spectrometry, microelectronics, and bioinformatics, many new gene products and proteins are being discovered daily; however, a challenge exists in the experimental analysis of this massive amounts of data. Array-based integrated chips and micro-fluidics hold a great potential for the development of high-throughput approaches to systematically analyze these proteins and to assign a biological function, determine protein–protein and protein–DNA interactions. These proteins can be robotically arrayed to generate protein chips, and each protein spot can be addressed by other proteins to determine recognition events and kinetics. Soft lithography [115] and microcontact printing [116] are potentially high-throughput

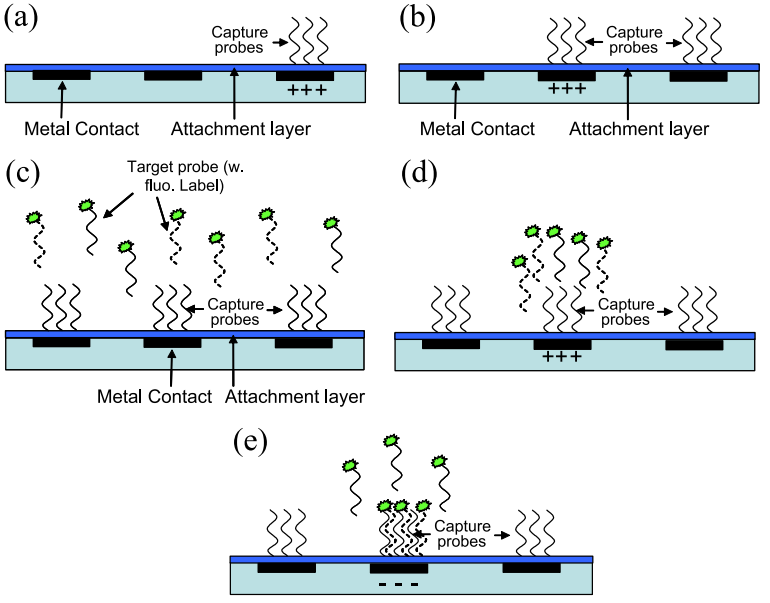

Fig. 11. Electric field mediated synthesis of DNA micro-arrays. (a, b) Capture probes can be sequentially addressed at specific sites, (c) target probes and label are added, (d) voltage applied at specific sites increases the local concentration and hybridization is performed, (e) the unhybridzed strands are repelled away.

and low-cost techniques that can be used for the printing of these arrays with high spatial resolution and ease. The binding has traditionally been detected by fluorescence-based methods, but it can also be detected by changes in surface refractive index such as in the BIACORE, surface plasmon resonance [117,118], or immunologically [119] on chip surfaces for high-throughput analysis.

3.3. Lab-on-a-chip and micro-fluidic devices

Lab-on-a-chip is another term used for μTAS and is used to describe sensors and devices with some level of integration of different functions and functionality. These devices offer the advantages of integrating sample handling and preparation, mixing, separation, lysing of cells, and detection. Many of these devices include more than one step of analysis, for example, sample preparation and detection, cell lysing and PCR, cell growth and detection of metabolites, etc. Numerous examples of such integrated devices and lab-on-a-chip have been reported for the processing and detection of cells, proteins, DNA, and small molecules. For the case of cells, a schematic of an integrated systems with all functions needed is shown in Fig. 12. All functions shown in this schematic might not always be used, rather only some of these may be integrated to achieve a specific aim. For example, for the case of DNA detection, the cells might be lysed and then an on-chip PCR device might be used to perform amplification and detection using specific primers. On-chip ELISA-type assays might require selective capture using antibodies immobilized on micro-fabricated surfaces, coupled with electrical or optical detectors. On-chip micro-capillary electrophoresis can be used to separate chemicals and different analytes. Many of the sensors described earlier form essential components of lab-on-a-chip. Recent reviews of lab-on-a-chip for drug development and cellomics applications have been presented [17,120,121]. Since the reduction of the channel diameter results in better separation performance and shorter channel length results in shorter transport time for electrophoretic separations, construction of a miniaturized 'total chemical analysis system' was proposed more than a decade ago [122,123]. Since then, this miniaturization has been demonstrated using silicon chip technology by a number of researchers. Glass micromachining was used to fabricate chemical analysis systems on chips that used electroosmotic pumping to drive fluid flow and electrophoretic separation to distinguish sample components with no moving parts [124]. Pharmaceutical compounds can be rapidly evaluated using these miniaturized devices on silicon and glass substrates [125]. DNA detection in nano-liter size samples using a device with integrated fluidic channels, heaters, temperature sensors, and fluorescence detectors has been described, as

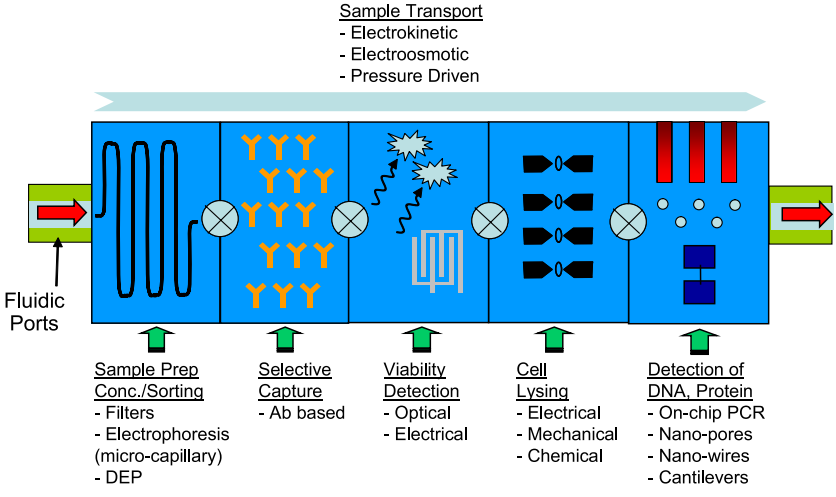

Fig. 12. Possible integrated platform for a lab-on-a-chip for detection of cells and microorganisms. Various modules could be used in appropriate combination for the detection of desired entity.

shown in Fig. 13 [126]. The device was reported to be capable of measuring aqueous reagent and DNA-containing solutions, mixing the solutions, amplifying or digesting the DNA to form discrete products, and separating and detecting those products, using on-chip capillary electrophoresis. The fluorescence detection was performed with on-chip photo-diode detectors. Many of these devices are being developed for one-time use assays (to prevent cross-contamination) and, hence, the use of plastic biochips is very prevalent. Disposable plastic fluidic biochips have been developed with on chip air pressure sources for fluidic movement and electrochemical detection of metabolic parameters for point of care health monitoring applications [127] and using magnetic-bead based biodetection of DNA and proteins [128,129]. Micro-mixing, flow sequencing, and metering using balanced centrifugal and capillary forces in CD-type plastic biochip has been described, as shown in Fig. 14 [130]. Such devices are very attractive due to their low cost, CD-type format, and integration with available optical detection technology. This technology has also been applied to detection of ions using

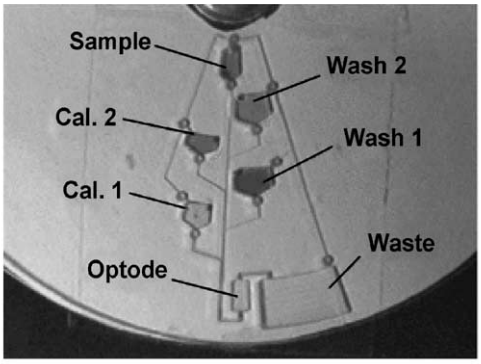

Fig. 14. Micro-fluidic devices on a CD type platform using centrifugal and capillary forces for liquid transport Madou et al. [130]. Reprinted with permission from Biomed. Microdevices 3 (3) (2001) 245–254 and with kind permission from Marc Madou.

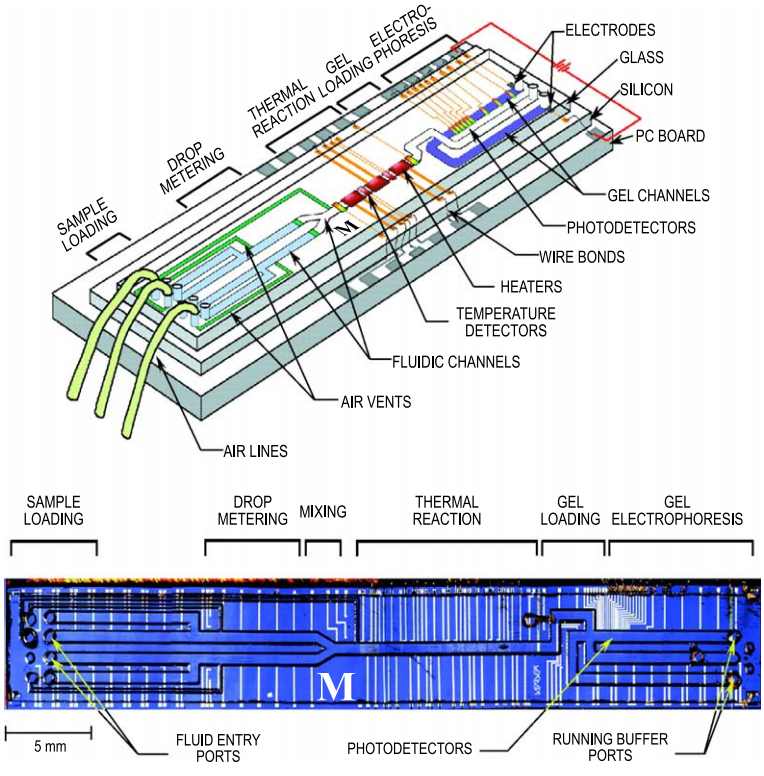

Fig. 13. Schematic of an integrated nano-liter DNA analysis device with various modules integrated into one device Burns et al. [126]. Reprinted with permission from Science 282 (5388) (October 16, 1998) 484–487 and with kind permission from Mark Burns.

ion-sensitive optodes integrated onto CD-based biochips [131]. Whole-wafer micro-fabricated capillary array electrophoresis DNA detection systems made in silicon have also been demonstrated here the capillary channels are made along the radius of the wafers [132,133]. Fully integrated genomic analysis microsystem including microfabricated heaters, temperature sensors, and PCR chambers have been demonstrated to successfully determine the sex from human genomic DNA in less than 15 min [134]. The PCR chambers are directly connected to the gel-filled capillary electrophoretic separation channels, where the voltage is applied using on chip patterned electrodes. High-throughput chemical analysis of cells has also been demonstrated in plastic biochips using hydrodynamic transport of cells, electric field mediated lysing, and fluorescence detection (off-chip detectors) at an analysis time of about 10 cells/min [135]. Fig. 15 shows an image of the biochip used for analysis of cell lysates in this study. Polymer and silicon devices have also been fabricated for the growth of bacteria and for their rapid detection within micro-fluidic devices [136,137]. Sample preparation and DNA extraction for use in micro-fluidic biochips [138] is also a very important module to be integrated in such lab-on-a-chip opportunities for integrated electronic detection of cell lysates, DNA, mRNA, and cellular proteins from just a few cells still remains outstanding.

As mentioned earlier, polymer and hydrogel-based micro-devices have many attractive features for use in biomedical lab-on-a-chip applications such biocompatibility [9], low cost combined with rapid prototyping techniques [11,139], and micro-fabrication of polymers [140]. Scaling down of the hydrogel features to produce self-regulating structures with response time of less than 10 s within micro-fluidic channels has been shown [141–143]. These photo-definable polymer approaches simplify the device fabrication and provide means to sense and actuate and can form important components of autonomous micro-total analysis systems.

It should also be mentioned that many important components of an integrated lab-on-a-chip have been reported elsewhere and are under development. These include valves, metering element, cell lysing elements, mixers, micro-pumps, etc., and a large body of literature exists describing the development of these elements. In addition, the very important topic of micro-fluidics [144], and integration of electrical (electrophoresis, dielectrophoresis, electroosmosis) and optical (laser tweezers, etc.) signals with micro-scale flow for manipulation and transport of biological entities [145–147] are also not dealt with in detail in this review.

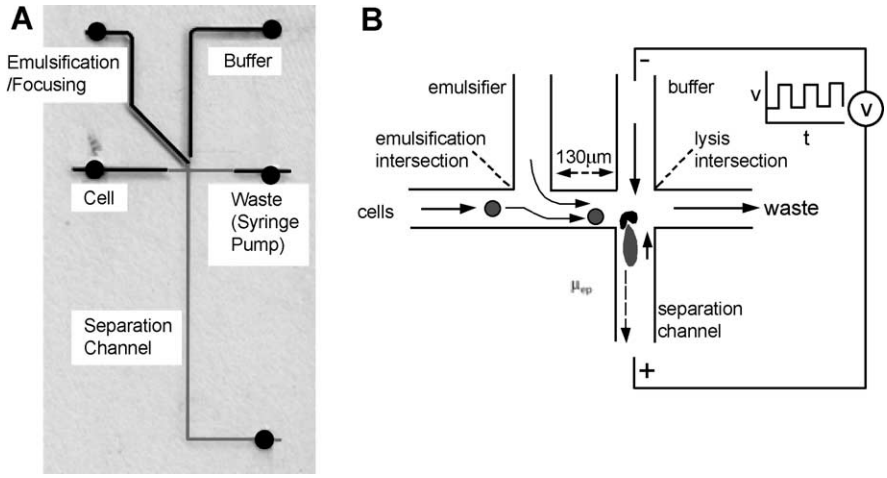

Fig. 15. Plastic biochips using hydrodynamic transport of cells, electric field mediated lysing, and fluorescence detection (off-chip detectors) at an analysis time of about 10 cells/min McClain et al. [135]. Reprinted with permission from Anal. Chem. 75 (21) (November 1, 2003) 5646–5655 and with kind permission from M.J. Ramsey.

4. Conclusions and future directions

Considerable progress has been made in the field of BioMEMS, some described above, and the research areas now merge and integrate into nanobiotechnology [148]. The commercial examples of BioMEMS and biochips, including micro-fluidics, continue to rise steadily. Just like MEMS are now considered as the technology to interface the macro world to the nano world, BioMEMS will also enable us to probe, measure, and explore the nano-machinery in the biological world such as single cells. Lots of great discoveries are anticipated in these exciting research areas, some possible future research directions and possibilities are briefly listed below.

4.1. Integrating diagnostic with therapeutic devices and personalized medicine

Significant strides have been made towards developing highly sensitive and integrated devices for sensing as described earlier. Challenges and opportunities still exist in the area of continuous monitoring and early detection of clinically significant proteins directly from blood and other body fluids. Detection of cancer markers, for example, can help millions to detect different forms of cancer before it is too late. The challenges of developing miniature sensors where the sensing surfaces can be regenerated, are biofouling resistant, and can be used for long periods of time in vivo are yet to be fully overcome. For in vitro sensors, the issues of rapid time along with highly detection is still outstanding. The century of personalized medicine will require rapid detection technologies that will provide the health care providers with genetic differences and variations between individuals to be able to personalize the health care.

Much progress has also been made in therapeutic micro- and nanotechnology (reviewed elsewhere, e.g., Ref. [149]). Some specific examples include (i) silicon-based implantable devices that can be electrically actuated to open an orifice from which pre-loaded drugs can be released [150], (ii) silicon devices functionalized with electrically actuated polymers which can act as a valve or muscle to released preloaded drugs [151], (iii) silicon-based micro-capsules with nano-porous membranes for the release of insulin [152], (iv) all polymer (or hydrogel) particles which can be preloaded with drugs and then forced to expand upon exposure to specific environmental conditions such as change in pH and release the loaded drug [153], (v) metal nano-particles coated with recognition proteins, where the particles can be heated with external optical energy and can locally heat and damage unwanted cells and tissue [154], etc. The possible integration of these and other types of therapeutic micro/nano-scale technologies with diagnostic devices for intelligent and integrated sensing and the ability to deliver known types and quantities of stimulus, drugs, and chemicals would be highly beneficial. The power source for such an integrated device is an important consideration and the goal is to have an autonomous device requiring little or no external power. Fig. 16 shows a concept schematic of such an integrated device with the various functional elements needed.

4.2. BioMEMS for hybrid devices and 3-D artificial organs

Tissue engineering for the realization of parts of or whole artificial organs is a very important and challenging area of research [155,156]. The development of hybrid artificial organs that utilize some inspiration

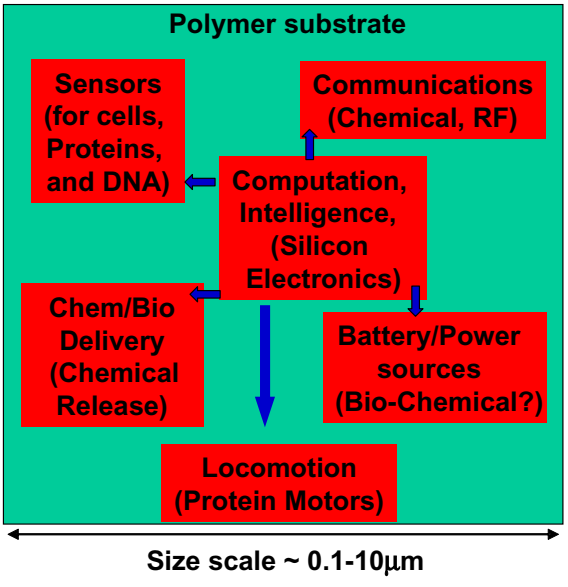

Fig. 16. Schematic of components needed for autonomous integrated diagnostics and therapeutic devices.

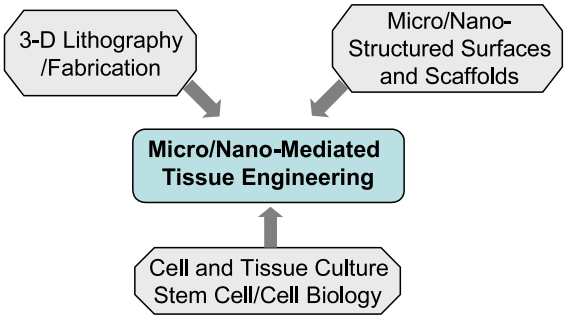

Fig. 17. Opportunities in micro/nano-mediated tissue engineering.

from micro or nano-scale technology is now also a very promising area of research [13,157–159]. PDMS-based microstructures have been explored for their use as scaffolds for cell and tissue engineering [160,161]. Three-dimensional structures composed of hydrogels with living human hepatoma cell lines were developed using photo-patterning techniques [162]. The formation of biocompatible polymeric scaffolds of specific shape, surface properties, and ability to promote cell adhesion and growth is a challenge, and the goal of these studies was to form such scaffolds using micro-fabrication techniques. It is well-known by biologists that small tissue samples and cells placed next to each other can fuse and form functionally active organoid structures. Examples of this include the development of sheets of myocardial cells, without a scaffold [14]. Electrical communication established between different layers of the myocardial cells demonstrated by autonomously beating of the stack of layers. Modified desktop inkjet printers filled with cells and a biocompatible ink system [163,164], three-dimensional thin layers of alternately printed cells were deposited, which initially formed clumps and later fused into vascular structures [165]. An essential component of this setup was the use of a thermoreversible and biocompatible gel that was liquid at 20 °C and solid at 37 °C. Given that the formation of vascular structures in artificial organ replacements is a very challenging task, these rapid prototyping approaches promise significant rewards in the tissue engineering field. As schematically shown in Fig. 17, using a possible combination of stereo-lithography [166], ink-jet printing of cells and the extra-cellular matrix on curved biocompatible surfaces, appropriate cell signaling and differentiation methodologies, and micro/nano-structured surface control, the development and construction of artificial organs can be a very exciting and fruitful area of research.

4.3. BioMEMS for novel tools in nanobiology

BioMEMS hold a lot of promise for the analysis of single cells and the study of their function in real time. Micro- and nano-scale systems and sensors could allow us to precisely measure the protein, mRNA, and chemical profiles of cells in real time, as a function of controlled stimulus and increase understanding of signaling pathways inside the cell. These are essential to increase our understanding of the underlying cause of basic cell functions such as differentiation, reproduction, apoptosis, etc., and their implications on various disease states. These issues will also be the focus of the post-genomic era and also in the applications of systems theories to biology, also referred to as systems biology [167]. To accomplish these goals, BioMEMS can play an important role, especially in the development of integrated devices and systems for the rapid and real-time analysis of cellular components, specially from single cells. Current expression analysis is performed from an aggregate of cells, lysed at specific time points when the mRNAs are analyzed. The development of micro-environments, as schematically shown in Fig. 18,

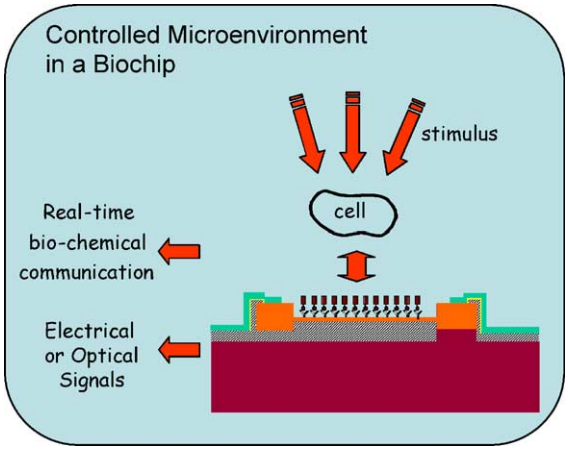

Fig. 18. Micro-fluidic devices with controlled micro-environments for study of cells and the real time profiling of their proteins, mRNA, and other biochemicals.

where cells can be precisely place, manipulated, lysed, and then analyzed using micro- and nano-sensors in 'real-time', would have a significant impact on systems biology. Integration of sensors for detection of DNA, mRNA, proteins, and other parameters indicating cellular conditions such as oxygen, pH, etc., can be accomplished using BioMEMS platforms and nano-scale sensors. These goals are now being pursued by many groups across the world.

Another very exciting research area where novel tools at the micro- and nano-scale can play an important role is in the area of Synthetic Biology, which can be defined as the re-design, fabrication, and alteration of existing biological systems, or design and fabrication of biological systems and sub-systems that do not exist yet (see Science, vol. 303, 9th Jan, 2004, p.158). The specific examples of this interdisciplinary field have recently been in the area of genetically engineering bacterial cells towards the goals of building digital networks. A bacterial oscillator was built using a network of three genes, which was inserted into *E. coli* cells to form a blinking oscillator [168]. Bacterial genome can be altered using recombinant DNA technology and microorganism can be constructed, potentially, to harness energy, decompose toxic waste, and possibly perform computational functions. As the field progresses, there will be a need for tools and technologies to perform gene insertions into single or very few bacteria, to specifically manipulate their characteristics within a network of bacteria. The tools and platforms to perform such integrated synthetic biology can be provided by BioMEMS and related nano-scale sensors, processing, and device technologies.

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