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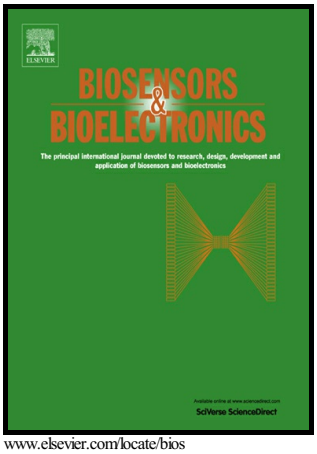

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PII: S0956-5663(16)30034-3
DOI: http://dx.doi.org/10.1016/j.bios.2016.01.033
Reference: BIOS8373

To appear in: *Biosensors and Bioelectronic*

Received date: 5 December 2015
Revised date: 11 January 2016
Accepted date: 12 January 2016

Cite this article as: Burak Derkus, Applying the Miniaturization technologies fo biosensor design, *Biosensors and Bioelectronic* http://dx.doi.org/10.1016/j.bios.2016.01.033

Applying The Miniaturization Technologies for Biosensor Design

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Abstract

Microengineering technologies give us some opportunities in developing high-tech sensing systems that operate with low volumes of samples, integrates one or more laboratory functions on a single substrate, and enables automation. These millimetric sized devices can be produced for only a few dollars, which makes them promising candidates for mass-production. Besides electron beam lithography, stencil lithography, nano imprint lithography or dip pen lithography, basic photolithography is the technique which is extensively used for the design of microengineered sensing systems. This technique has some advantages such as easy-to-manufacture, do not require expensive instrumentation, and allow creation of lower micron-sized patterns. In this review, it has been focued on three different type of microengineered sensing devices which are developed using micro/nano patterning techniques, microfluidic technology, and microelectromechanics system based technology.

Keywords: Miniaturization, Microtechnology, Nanotechnology, Micropatterning, Nanopatterning, Microfluidics, MEMs

1. Introduction

As of the date of 1953, after development of the first electrode by Clark for biological purposes, biosensor technology has grown rapidly, these courses of events continue even today. Advances in biosensor technology can be investigated considering two components: The advances in biological and physical parts. To understand the growth rate, the types of biosensors can be investigated in biological manner. Following enzyme sensors, immun sensors (Yao et al., 1987, Derkus et al., 2013, Derkus et al., 2014), hormone sensors (Vijayen et al., 2015, Johansson and Hellenas, 2001), cytosensors (Wu et al., 2012, Sun et al., 2016),

and DNA sensors (Kung et al., 1990, Rashid et al., 2014) have become popular at short intervals.

In the early 2000's, development of microtechnology gave the biosensor technology a new point of view. Microscale engineering plays an important role in design of tools for biological applications by scaling down systems and providing controllable microenvironments. Among these, microfluidic systems may be the most studied and well defined systems compared to microelectromechanic systems (MEMs) and other micron sized devices. Micro-systems offer various advantages in comparison to traditional ones. The most important advantages are allowing lower use of expensive materials such as biological agents, manipulation of the environment, integration of various seperated parts on a common substrate, working multiple simultaneously, and ability to accurately control fluid flow with the use of peristaltic pump or another automatization system. Specifically in sensor technology, microscale environment eliminates some disadvantages such as non-uniform pH distribution, electrical distortion, non-homogenous application of electrical perturbation by controlling the spatio-temporal variations. Some optical detection methods, that cannot be easily applied to traditional sensing systems, such as fluorescence lifetime-imaging, time-lapse microscopy, multicolor analysis are successfully used as measurment methods in microfluidic sensors (Behninger et al., 2007, Hansen et al., 2015). Surface plasmon resonance (SPR), mass spectrometry (MS), and surface acoustic wave (SAW) are the other methods successfully utilized in microfluidic sensor design (Lazar et al., 2006, Galopin et al., 2007, Pohl and Weiss, 2014).

In this review, it was focused on recent efforts aiming the development of multifunctional, effective, practical, and innovative microfabricated sensing systems. Microfabricated sensing systems were investigated under the most popular three titles: Micro/nanopatterning for biosensor design, microfluidic biosensors, and MEMs based biosensors.

2. Micro/Nano Patterning for Biosensor Design

Miniaturizing technologies provide many advantages in the development of diagnostic devices; low manufacturing costs, needs small quantities of samples, shortens the reaction time and different types of tests can be performed simultaneously on the same device. To meet these expectations, in the early 90's, microfabrication techniques rapidly took place in many fields of science such as molecular biology, bioengineering, medicine and pharmaceutical sciences. This process started with basic techniques than new methods which were consecutively developed in the course of time collected under the title "Photolithography". This technique mainly transfers geometric patterns from a photomask to a substrate such as glass or silicon wafer with UV (Ultra Violet) exposure. Photolithography consists of several steps including pattern designing, printing the pattern on to a photomask, spin coating of substrate with photoresist which acts as master, transferring the pattern from the mask to the substrate with UV exposure, pouring the polymer, getting the mold, and baking, respectively.

Soft lithography is the most commonly used technique for the fabrication of micropatterned materials (Kane et al., 1999, March et al., 2015). Designed patterns are generated onto silicon wafers using UV light sensitive polymers. The characteristic material of this method, PDMS (polydimethylsiloxane), is poured onto the patterned master and is peeled off after crosslinking reaction has finalised. PDMS attracts attention with its various superior features such as non-toxicity, biocompatibility, flexibility, allowing easy peeling from master mold, transparency particularly important for optical devices, and cheapness.

Figure 1 is here

In a study carried out in 2011 by Lee et al., nanofiber-based protein microarray fabrication using two popular techniques, electrospinning and hydrogel lithography, was demonstrated. In

the aformentioned study, hydrogel microstructures were created using polystyrene (PS)/poly(styrene-alt-maleic anhydride) (PSMA) composite fibers with diameters ranging from 0.5 to 1.0 mm prepared by electrospinning in combination with photopatterned poly(ethylene glycol) (PEG). The resultant microgels have an advanced IgG (Immunoglobulin G) immobilizing feature thanks to protein-repellent nature of PEG hydrogels which enables creating an effective IgG microarray (the macroscopic image can be seen in Figure 1A) and thus detecting proteins sensitively. When the results were investigated, enhancement of the fluorescent signal as a consequence of advanced IgG immobilizing feature (6 times higher when compared to planer array) of microgels due to their increased surface was seen.

Nanoimprint lithography, another lithography technique, is one of the most promising technologies for fabricating microsize to nanosize patterns with advanced resolution and low cost, speedy production compared to other lithography methods (Ohtake et al., 2006, Eom et al., 2015, Song et al., 2016). This technique is mainly based on pressing a transparent stamp with a defined pattern onto a thin resin layer at mild conditions and pressure. The resin is then treated with UV light and separated from the patterned layer. Finally, the patterns are transferred to the desired substrate using etching techniques, followed by metal deposition and removal of the remaining resin (Guo, 2004). Using this technique, Lee et al. (2011) fabricated an elliptical gold nanodisc array as a localized surface plasmon resonance (LSPR) sensing substrate for clinical immunoassays. In this study, the first example of an enzyme-substrate reaction to a sandwiched immunoassay-based LSPR biosensor was demonstrated which aside the disadvantage of short penetration depth of the plasmon field and thus enables high levels of sensitivity. The authors indicates that this phenomenon is effective more when large-sized antibodies are used as bioreceptors. Using this technique, picomolar levels of prostate specific antigen (PSA) could be detected which is far lower than physiological levels of PSA (~5

ng.mL^{-1}) and also 10^5-fold lower than the label-free detection without the enzyme precipitation.

Stencil lithography is, on the other hand, a relatively novel technique based on shadow mask evaporation in which stencils, thin layers of materials including holes with different sizes and shapes, are used for patterning (Arcamone et al., 2009). Using this technique, various nanostructures such as nanowires (Brugger et al., 2000, Villanueva et al., 2008), nanodots (Tun et al., 2007, Vazquez-Mena et al., 2008, Villanueva et al., 2010) and plasmonic structures (Aksu et al., 2010, Vazquez-Mena et al., 2010) could be produced using suitable materials for sensing technologies, especially for plasmonic biosensing. Compared to traditional methods, stencil lithography has some unique properties such as not requiring resist coating, baking, or any lifting-off steps. Another advantage of stencil lithography is the reusability of stencils for several times and thus low-cost production. Best to our knowledge, there are only a few studies on biosensors developed using stencil lithography, one of which was carried out by Vazquez-Mena (2010) for the detection of streptavidine using SPR (Vazquez-Mena et al., 2011). In this study, arrays of 50-200 nm wide nanodots with different spacing of 50-300 nm are fabricated without any resist, etching, or lift-off processes (Figure 1B). The fabricated nanodots show localized surface plasmon resonance in their extinction spectra in the visible range and the resonance wavelength depends on the properties of the nanodots. With this technique, streptavidin could be successfully detected in level of 100 nM.

Biosensor design studies using micro/nanopatterning technologies are limited with only a few papers. Some other patterns, such as circular (Figure 1C), spot (Figure 1D), and nanodome (Figure 1E,F), have been also reported in the literature, of which details have been presented in Table 1. In case being advances in patterning technologies, more intelligent and useful systems are expected to be designed in the future. These techniques can also enable the design

of functional sensor systems towards to single cell analysis and useful microarrays which have the capability of simultaneous detection of many biological molecules.

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Table 1 is here

3. Microfluidic Biosensors

Figure 2 is here

Immediately after the first micro-components for fluid manipulation such as micropumps, microvalves and micromixers were developed, an innovative idea, microfluidic technology, was put forward by scientist combining both capillary flow and microfabrication techniques (Manz et al., 1992). Early microfluidic devices included only microchannels, and the other micro-components joined them in the course of time as represented in the timeline in Figure 2. Nowadays, complex microchips with desired properties for various purposes such as diagnosis (Garcia-Cordero et al., 2014), isolation (Hyun et al., 2013), photodynamic therapy (Lou et al., 2014), tissue engineering (Buchanan et al., 2014), and stem cell bioengineering (Shamloo et al., 2015) can be easily fabricated. Microfluidic devices have some unique features such as bearing micro-channels down to nanoliter volumes, which can also act as a bioreactor and thus decrease analysis time from minutes to seconds. The portable nature of these devices enables the development of easy-to-use and cheap diagnostic tools which are important parameters for commercialization. Microfluidic devices are fabricated using soft lithography techniques as described in the "micro/nano patterning for biosensor design" section. Figure 3 represents some microchip designs towards sensing of different kind of biologicals.

Figure 3 is here

Some important functions of microfluidic systems used for biological analysis are sample manipulation, effective and rapid reaction, and detection. Besides detection and reaction, that

are improtant in all cases, sample manipulation is particularly important for microchips which use whole biological samples. This manipulation is generally carried out by means of microvalves, micropumps, or micromixers. A good example for microfluidic biosensor design including sample manipulation has been reported by Lee et al. (2009). The microchip proposed in this study integrates micropumps, a four-membrane-type micromixer, an on-chip microcoil array that allows simultaneously detection of IgG and IgM, and thus enables the confirmation of dengue virus infection (Figure 4A). The main mission of the coil array here is direct current triggered bio-separation of IgGs, which are prepared or mixed ina micromixer of which the working principle is based on air compression. For the purpose of use in separation and extraction of plasma from whole blood using dielectrophoresis, Szydzik et al. (2015) have developed a microchip that has a main channel and a cross flow filtration system for extracting plasma from whole blood (Figure 4B). Requirement of external pumping or dilution component, which decreases performance of the manipulation and leads to lower concentration of target, makes the traditional methods less effective. Therefore, such capillary-driven, dielectrophoresis-enabled microfluidic systems thought to be useful for obtaining cell-free plasma using only a small amount of whole blood. Batalla et al. (2015) have utilized from electro-injection and electro-focusing for sample manipulation (Figure 4C). The microfluidic chip designed for the separation and enantiomeric detection of D-Methionine (D-Met) and D-Leucine (D-Leu) consists of enzymatic buffer reservoir, amino acid reservoir, and detection reservoir. Using this multi-functional chip, they could achieve the successful separation and detection of D-Met and D-Leu. In literature, there are also some microchips including microreaction or microdetection chambers, wherein a biological reaction occurs or detection by means of a transducer is carried out. Chang et al. (2015) have designed a microchip for rapid detection and typing of live bacteria from human joint fluid samples (Figure 4D). The microchip has various manipulation parts such as micropumps,

microvalves, suction units, or Polymerase Chain Reaction (PCR) chamber where the bacteria typing are performed. This kind of design provide the clinicians opportunity of simultaneously PCR detection using desired antibiotics. To improve the biological reactions in microdevices, Cohen et al. (2015) have provided a different microfluidic approach. The proposed design integrates microspheres that enable dynamic detection of the analyte, Tumor Necrosis Factor-α in this study, by continuously sampling micro-liter volumes of sample per minute to detect dynamic changes in target analyte concentration (Figure 4E).

Figure 4 is here

As can be understood from all these works, one of the most important goal of the researchers in microfluidics field is to improve sample manipulation and reaction efficiency, hereby offer the clinicians helpful systems for the early, cheap, simultaneously, and rapid diagnosis of various disease.

PDMS is the most widely used polymer for the fabrication of microfluidic devices (Mohan et al., 2013, Zhi et al., 2014, Zhang et al., 2014, Zilberman et al., 2015). Scientists have started to discover and manipulate novel polymers for the fabrication of microfluidic devices with a more efficient fluid delivery, and development of micro-components. One of these is the porous polymer monolith (PPM) of poly(N-isopropylacrylamide) (PNIPAAm) studied by Xiong et al. (2013) as a new strategy for replaceable enzymatic microreactor based on a switchable wettability interface for the purpose of glucose detection. PNIPAAm has a temperature-responsive structure characterized by low critical solution temperature (LCST) of 32 ^{0}C due to hydrogen-bonding interactions of the amide group. This smart polymer is swollen and hydrophilic when its temperature is below LCST, whereas it is shrunken and hydrophobic when its temperature is above LCST. Considering these thermo-responsive properties, replaceable microfluidic enzymatic biosensors have been designed by means of

reversible adsorption and release of glucose oxidase (GOx) on the robust and stable matrix and employed for detection of glucose with a linear range from 0.05 to 5 mM.

In a study carried out by Roy et al. (2011), styrenic thermoplastic elastomers have been preliminarily used for the fabrication and assembly of pneumatically driven micro-valves in a microfluidic device. This three block (styrene)–(ethylene/butylene)–(styrene) thermoplastic co-polymer is as transparent, biocompatible and flexible as PDMS, and is suitable for industrial applications, a shortcoming of PDMS based processes. The other superior features of this polymer are no pre-requiring vulcanization steps like in PDMS, allowing free pressure molding, and being an appropriate material for bonding and assembling.

Malic et al. (2013) have designed a nanoplasmonic microfluidic device using the (styrene)–(ethylene/butylene)–(styrene) thermoplastic three block co-polymer. The microchip consists of a flow layer in a hard cyclo-olefincopolymer (COC) thermoplastic substrate and has been designed as 8 x 8 array including gold coated nanostructures enabling SPR, a control layer prepared with the three block co-polymer, and a thin thermoplastic elastomer membrane sandwiched between the two. An important feature of the bottom material is allowing integration of active microfluidic components, such as pneumatic valves. Picomolar levels of sCD44 protein, a cell surface glycoprotein, could be detected using transmission SPR techniques with the newly developed microfluidic immunosensor.

Another polymer that has been alternatively used for fabrication of microfluidic elements is pH-stimuli polysaccharide chitosan. Luo et al. (2010) have used this polymer to form a hydrophilic membrane structure with a pore size of a few nanometers in microfluidic networks where pH gradients are generated. With development of this kind of membrane, effective pumping could be produced in the device for fluid delivery as a result of deprotonation, and thus gelation and solidification.

Table 2 lists different microfluidic biosensing applications considering sensing method, a description of microchip, target molecule and detection limit. Considering this table, one can understand that electrochemical and optical methods dominate the big part of the studies, aiming the determination of biologicals using microfluidics. Microfluidic chips can be engineered to be suitable for the purpose, including lab-on-a-CD (Li et al., 2013), multi-layer chip (Matharu et al., 2014), power-free and portable (Ahmed and Azzazy, 2013), droplet-generating (shim et al., 2013), or multichannels for simultaneous detection (Cao et al., 2012). The common features of these chips are operating with low sample volumes, which vary between nano- or microliter, and having low detection limits as low as pico- or femtomolar (Sadabadi et al., 2013, He et al., 2013, Hunter et al., 2013, Malic et al., 2013, Shim et al., 2013).

Table 2 is here

The follow-up studies focused on "optofluidics" sensing systems, a relatively young research area, which combines microfluidic technology with optical transducing methods, and thus makes it possible to create an innovative class of sensors. Optofluidics concept aims to build up novel optic, mostly photonic, materials and to integrate them with micro/nano fluidic systems. Unlike electrochemical measurement techniques, optical methods are compatible with some biological solutions and are not affected from ionic properties of measurement enviroment. The use of optic waveguides in microfluidic designs enables an efficient optical interaction with target molecule and hence increases sensitivity. In a study carried out by Yanik et al. (2010), a plasmonic nanohole array has been developed towards to design of an optofluidic sensor for the detection of small enveloped RNA viruses (vesicular stomatitis virus and pseudotyped Ebola) and large enveloped DNA viruses (vaccinia virus). Electron beam lithography together with direct deposition of metallic layers have been used to form the hole patterns, that eliminates the disadvantages of lift-off nanofabrication process. Following

the formation of the patterns, the holes have been covered with a photonic crystal-like free-standing SiN*x* membrane. When the antibody modified optofluidic sensor has been incubated with virus containing sample, a red-shifting (~100 nm) of the plasmonic resonance peak has been observed as a result of interaction of virus and antibody. Using the proposed sensor, a dynamic range of 10^6 to 10^9 PFU/mL virus has been obtained as a consequence of calibration study.

Chung et al. (2015) has developed an optofluidic system that recognizes fluorescence nanoparticles conjugated with aptamer chains specific to E. Coli. In this real-time and continuous single cell detection system, fluorescence nanoparticles bind to bacterial cell surface to make them distinguishable when they excited with wave-length of 540 nm. The developed system consists of T-shaped PDMS microchannel which has 1 sample flow and 2 sheath flow inlets, and 1 outlet, and an optical detection part. Using this optofluidic system, 100 particles per second could be detected with high accuracy (85%).

In a Follow-up work, Ozcan et al. of University of California have focused on improving the performance of optofluidic systems. In a study carried out by Ozcan Lab, an opto-electronic (Metal–Oxide–Semiconductor, CMOS) imager part to record the diffraction patterns of plasmonic nanoapertures has been integrated with a basic microfluidic chip for lens-free sensing of proteins (Coskun et al., 2014). In this study, a novel nanofabrication method, deep UV lithography, has been firstly used to form nanoholes, in other words biosensor pixels for this study, which has some advantages such as enabling to rapid fabrication when compared to electron beam lithography. When plasmonic materials are used in combination with lens-free imagers, multiple nanoplasmonic structures can be easily analyzed simultaneously. Interaction of the target proteins with their ligand-proteins modified onto nanoplasmonic structure causes a spectral shift in the peak wavelength of the plasmonic mode. Using this

lens-free nanoplasmonic optofluidic sensor, 12.5 picomoles per minute of IgG has flowed through the chip for the protein detection experiments.

4. Microelectromechanics System (MEMs) Based Biosensors

MEMs offer some great opportunities compared to other sensor systems due to their small dimensions, electrical nature, and ability to operate on short time scales. Image processing for prothesis (Xia et al., 2015), histological monitoring (Duan et al., 2015), flexible electronics for neural recording (Xiang et al., 2015), and drug/vaccine delivery (Pirmoradi et al., 2015) can be counted among the potential fields of MEMs, besides biosensors that utilize MEMs technology. In MEMs, mechanical parts such as sensors, actuators, or electronics are inegrated on same substrate, generally on a piece of silicon, using microfabrication techniques. Thus, a more practical and economic, less power consuming or self-power generating, and miniaturized diagnosis devices can be designed. Another important feature of these kind of devices is delivering the electrical output directly. This is significant because they remove the requirement of an additional part providing the signal transduction and background noise as well. When the papers are investigated within this scope, a considerably few study is seen, most of which are towards to development of implantable glucose sensor.

Instead of assuring blood in each time, subcutaneously implantable biosensors are useful tools to measure blood glucose levels. Combining the implantation mentality with affinity glucose sensing technique, Huang et al. (2013) has developed a MEMs based continuous glucose monitoring system, in which affinity sensing method enables a long-term and stable glucose management. This capacitive-based system, formed between a pair of parallel electrodes, contains no moving parts, an important feature for implantable systems. The developed MEMs sensor consists of three main parts: Perforated upper gold electrode, a gold electrode lying on the substrate, and a perforated diaphragm providing stability against in vivo

mechanical disturbances. The working principle of the proposed system relies on the affinity binding of the polymer solution, that takes place between the two gold electrodes and also acts as an electrolyte, with glucose. Decrease in the capacitance occurs as a consequence of this binding event. 83 ppm of capacitance deviation and 2.5-3 min of time constant obtained as a result of glucose concentration change from 60 to 120 $mg.mL^{-1}$, and then 60 $mg.mL^{-1}$ again. These data show the stability and practical usability of the sensor system. These kind of systems may thought to be promising candidates for the monitoring of glucose level *in vivo*.

Another important area in which MEMs based sensors can be used is diagnosis and monitoring of cancerous tissues, and distinguishing tumor tissues from healthy ones. For the purpose of distinguishing the malign breast tumor tissue from the benign one, Pandya et al. (2015) has developed a flexible MEMs based electro-mechanical sensor array that is bending- or force sensitive. The sensor array consists of eight strain gauges that act as mechanical sensors and have been fabricated using conductive polymers poly(3,4 ethylenedioxythiophene) and poly(styrenesulfonate) (PEDOT:PSS) on poly(dimethylsiloxane) (PDMS) as well, that gives a transparency. The pillar shaped sensors have been fabricated using SU-8 photoresist polymer on Au pads that resides on 1 μm thick SiO_2 (silicone dioxide) layer. The pillars are then coated with Au to gain conducting property. Thus, the pillars could be used for two purposes: For transferring the force to strain gauge (mechanic), and as conductive probe for the monitoring of electrical change. When a force alteration occurs on the pillars, the electrical properties of the film change due to the piezoresistive effect. To measure electro-mechanical change, a definite force is applied by means of AFM (Atomic Force Microscopy) on the pillars that results in the bending of the strain sensor and corresponding changes in electrical properties are calculated. Using this flexible MEMs sensor, breast cancer tissues can successfully be distinguished from benign tissue based on an electro-mechanical approach.

In another study, Timurdogan et al. (2011) has developed a MEMs based sensors towards to detection of Hepatitis A and C viruses in serum samples (Figure 5A). For this purpose, they have used a microcantilever type sensor which they have presented in a previous work (Ozturk et al., 2008). The fabrication process of the proposed MEMs sensor is as follow: (i) Sputtering of Cr (Chromium,10 nm) and Au (Gold, 100 nm); (ii) formation of the functional pats (cantilevers and gratings) by means of photolithography, (iii) Au and Cr etching; (iv) Applying KOH (potassium hydroxyde) etching to remove the Ni (nickel) structures. An electromagnetic coil integrated to sensor system supplies the actuation, which makes the sensor system suitable for the operation in extreme conditions. On the other hand, the interferometric optical sensing has been carried out using laser illumination and has embedded diffraction gratings at the tip of each cantilever. For the recognition of Hepatitis antigen, related antibodies have been immobilized onto cantilevers. A resonant frequency shift occurs when the antibodies interact with the antigens, thus, sensing signal is obtained. Minimum detectable concentration limit for both antigens is 1.66 pM, that is quite low.

Yang et al. (2015) has developed a magnetoimpedance based microbial biosensor aiming the detection of Escherichia coli (E. coli) O157:H7 (Figure 5Ba). This microbial sensor has been prepared by applying the MEMs process, wherein Cr/Cu/NiFe/Cu/NiFe has been constructed using radio frequency magnetron sputtering, photoetching, and electrodeposition processes (Figure 5Bb-g). A self-assembled gold layer deposition has been also done to be used as immunoplatform, in other words, as antibody carrier surface. The fundamental principle for detection of E.coli O157:H7 is based on the interaction between the target bacteria and Dynabeads that are employed as magnetic labels of E. coli O157:H7, and thus E. Coli O157:H7 can be monitored by detecting the fringe field of Dynabeads using magnetic sensing elements (Figure 5Bh). Using this magnetoimpedance approach, a 100 cfu/ml of detection limit has been achieved for the target bacteria.

Kehl et al. (2016) has focused on improving the MEMs based sensors combining them with a relatively different transducing technique. This MEMs based biosensor includes micro-mirror to interrogate wave-guide grating regions in the kHz range by measuring the angle of light, that makes it a label-free optical system (Figure 5Ca). Adjustable micro-mirrors provid a wide scanning range, enabling detection of different wavelengths and optical powers. All of these features are important to reach lower detection limits and sensitivity (effective refractive index change is $< 2\times10^{-7}$). The working principle of this chip relies on the alteration of refractive index following the adsorption of target molecules on the sensor surface, that changes the coupling angle (θ_c) and can be measured by a temporally shifted intensity signal (Figure 5Cbc). When this MEMs microchip relying on a waveguide grating coupler logic and consisting of borosilicate glass substrate and a tantalum-pentoxide (Ta_2O_5) waveguide is investigated in terms of fabrication process, some extra steps such as reactive ion etching or Ta_2O_5 sputtering in addition to traditional photolithography are seen (Figure 4Cd-i). Using the proposed system, 2.5 nM of goat anti-mouse IgG as LOD has been determined.

Figure 5 is here

5. Conclusion and Future Outlook

At present, the fields of microtechnology and nanotechnology applied to health sciences are still in their infancy. Among all microfabricated sensing systems, microfluidic biosensors those are designed and produced easily, and generally do not include electrical or mechanical parts have the highest probability of making an impact in the near future. Being portable and economic could lead these systems to commercialization and to use extensively in human clinics in the next 20 years. Hovewer, these sensing systems have various lacks, some of which are easy production and successfully control of micromanipulators such as micropumps, microvalves, or micromixers. To eliminate these hurdles, easy-to-shape and -manipulate smart materials should be developed. Currently, these micromanipulators are

created during photolithographic process. In addition to this, external methods are necessary. Another lack is extensively application of these systems for clinical applications. To adapt these sensors to human clinics, the proposed systems should not be studied using only spiked serum samples but also be studied with real biological samples such as urine, whole blood, cerebrospinal fluid, or cancerous tissue homogenate. Telemedicine, that is an important and young working area nowadays, can provide some contributions to design portable and home-use biosensors when combining with microfluidics. Working principle of this lens-free technique relies on taking a photo of microfluidic chip in which biological sample injected. A software loaded to mobile phone calculates the level of the target marker or biological molecule and provides some information about the progress of the disease even. When this novel concept gets widely used, early diagnosis of various diseases will be possible.

Figure 6 is here

MEMs-based sensors have some disadvantages: The production process is difficult, they require expensive equipment, and it takes a long time to prepare these sensors due to including several coating or etching steps. Therefore, the studies towards to MEMs biosensors in the literature are limited compared to microfluidic biosensors. Today, researchers make major efforts for the development of MEMs-based microelectronics and their fabrication processes. For instance, Bhaskar et al. (2015) has developed a flexoelectric microelectromechanical system on silicon material, that allows polarizing in response to a mechanical bending moment, and bending in response to an electrical field (Figure 6A). This cantilever-like all-oxide construct has been fabricated as capacitor composed of a strontium titanate ($SrTiO_3$) active layer sandwiched between two layers of strontium ruthenate ($SrRuO_3$) for the top and bottom electrodes. The proposed lead-free flexible electromechanical actuator, that can be integrated onto silicon substrate for MEMs applications, is a promising candidate,

especially for capacitive biosensors. Similar approaches have been reported by Baek et al. (2011), Ghaysh et al. (2015), and Talugdar et al. (2015).

Successfully combining the integrated circuits (ICs) with MEMs would be another superiority for the development of biosensors, especially the electrochemical and optical biosensors, in addition to flexibility. Using traditional methods, MEMs and IC components are manufactured on separate substrates and are subsequently hybridized in the final system. Due to laborious of this method, researchers have discovered another technique, a system-on-chip (SoC) solution, wherein MEMs and ICs are manufactured on the same substrate (Fischer et al., 2015). Figure 6B presents a system-on-chip, based on polysilicon nanowire technology, towards to detection of Hepatitis B Virus DNA and cardiac troponin I protein (Huang et al. 2013). Still, the number of biosensor studies in the literature based on this innovative idea can be counted on the fingers of one hand (Pei-Wen et al., 2014). Last of all, further studies are needed to develop the MEMs based biosensor, and thus design high-performance portable biosensor systems.

Acknowledgments

This work was supported by the Scientific and Technological Research Council of Turkey (113S255).

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Figure Captions

Figure 1. Different patterns obtained for the design of sensing devices: **(A)** Micropillar shape, adapted from Lee et al. (2011) with permission from Royal Society of Chemistry. **(B)** Nanosquare, adapted from Vazquez-Mena et al. (2011) with permission from American Chemical Society. **(C)** Circular, reprinted from Lee et al. (2008) with permission from Elsevier. **(D)** Spot, adapted from Melamed et al. (2011) with permission from Royal Society of Chemistry. **(E)** Nanodome, adapted from Choi and Semancik (2013) with permission from American Chemical Society. **(F)** Whole sensing device including nanodomes, adapted from Choi and Semancik (2013) with permission from American Chemical Society.

Figure 2. Timeline of microfluidic technology. Dynamic micropump was adapted from Gerlach and Wurmus (1995) with permission from Elsevier. Droplet generator was adapted from Umbanhowar et al. (2000) with permission American Chemical Society. Pico and femtodroplet generator was adapted from He et al. (2005) with permission from American Chemical Society. Optopneumatic piston was adapted from Velez-Cordero et al. (2015) with permission from Royal Society of Chemistry.

Figure 3. Different microfluidic sensor designs: **(A)** Lab-on-a-CD system toward to whole blood analysis of glucose, lactate and uric acide. The system consists of motor, motor controller, CD panel, electrode leads (a), and potentiostat connectors (b). The microchip includes 3 layers: Top layer including microchannel, middle layer bearing 3D microvalve, and bottom layer including electrodes and contact pads (c). Adapted from Li et al. (2013) with permission from Royal Society of Chemistry. **(B)** Microfluidic device used for femtodroplet generation and manipulation toward to immunosensing. (a) Femtodroplet formation at the nozzle. (b) Apperance of the whole PDMS device. The upper layer consists of the nozzle, flow channels and storage compartments. The bottom layer includes the monolithic valves used to control droplet flow. When the main valve is closed, the stream of droplets is directed

into the storage region (stream path 1), whereas the droplets flow out of the device by stream path 2 when the valve is opened. (c) Layer-by-layer schematic presentation of the microchip. (d) Fluorescence images of femtodroplets containing anti-PSA-coated beads after incubation with PSA containing immunoassay. Adapted from Shim et al. (2013) with permission from American Chemical Society. **(C)** A multichanneled PDMS/glass/paper microchip. (a) The microfluidic system consists of two PDMS layers as top and middle layers and one glass plate as the bottom layer. The top layer has 32 micro-channels with100 μm wide and 100 μm deep and inlet reservoirs and a waste reservoir commonly used by each channel. The middle layer is the incubation layer which has a piece of chromotograph paper serving as the substrate for adsorbing the aptamer-functionalized graphene oxide in subsequent steps. The principle of the system based upon increasing of fluorescence as a consequence of interaction of fluorescently quenched fluorophore conjugated aptamer and the bacteria, the target analyte. (b) The fluorescence imaging of the microchip. Adapted from Zuo et al. (2013) with permission from Royal Society of Chemistry. **(D)** A bio-barcode assay type microfluidic chip toward to PSA detection. (a) The chip consists of two regions first of which seperates the target protein using the magnetic particles functionalized with monoclonal PSA antibodies and the second region, has a patterned complementary DNA, detects the barcode DNA released from seperation area. (b) The signal obtained from the gold nanoparticles, bounded to DNA, is amplified using silver stain. Adapted from Goluch et al. (2006) with permission from Royal Society of Chemistry. **(E)** Micro-a-fluidics ELISA chip for the detection of CD4 cells. The chip has five circular and five elliptical chambers which contain buffer and oil for seperation and washing steps. In chamber I, functionalized magnetic beads conjugated with anti-CD4 antibody are used to selectively capture CD41 cells. After a washing step in the intermediate chamber, the magnetic beads with captured CD41 cells are moved to chamber II and tagged with anti-CD3 antibody, which is conjugated with horseradish peroxidase. Finally, captured CD41 T lymphocytes cause a color development due to TMB by HRP in chamber III. The color development on-chip is imaged using a cell phone. Adapted from Wang et al. (2015) with permission from Nature Publishing Group. **(F)** Suction type microfluidic chip for the rapid measurement of dengue virus. General view of the microchip (a). The microchip consists of three chamber: Test, negative control and positive control chambers. The chip has 8 main inlet holes for dengue virus load (A), HRP-labled antibodies (B), washing buffer (C), tetra methyl benzydine (D), stopping buffer (E-G), internal positive control of IgG (H), and waste (I). Sequential fluid transport is seen in b-d. Using this pneumatically driven ELISA based microchip, 10 PFU/mL of dengue virus detection sensitivity was obtained. Adapted from Weng et al. (2011) with permission from Springer.

Figure 4. Different microfluidic biosensor designs including sample manipulation, reaction, or detection. **(A)** A microchip towards to dengue virus infection detection, adapted from Lee et al. (2009) with permission of Elsevier. **(B)** A microchip for seperation of plasma from whole blood. . (a) Top view, (b) real photo of the chip, adapted from Szydzik et al. (2015) with permission from AIP Publishing LLC. **(C)** Microfluidic platform that use electrokinetic and electro-focusing sample manipulation for seperation and detection of enantiomers. (a) Electrokinetic injection of enzyme and enantiomers, (b) electrofocusing of enzyme and aminoacids, (c) D-aminoacid seperation and enzymatic reaction, reprinted from Battala et al. (2015) with permission from American Chemical Society. **(D)** A microchip design including microvalve, micropump, and PCR reaction chamber for detection and typing of live bacteria from human joint fluid samples, reprinted from Chang et al. (2015) with permission from

Elsevier. **(E)** A different design enabling dynamic reaction study for TNF-α detection, adapted from Cohen et al. (2015) with permission from Elsevier.

Figure 5. MEMs based biosensors: **(A) (a)** Optic, and **(b-e)** SEM images of electro-mechanical sensor array. While **(b)** shows the general view of cantilever, **(c-e)** shows the SEM images of unmodified, antibody immobilized, and antigen incubated surface. **(f)** Appropriate flowcell for virus detection. **(g)** Illustration of the closed-loop broadband magnetic actuator and optical readout with integrated diffraction grating, reprinted from Timurdogan et al. (2011) with permission from Elsevier. **(B)** Magnetoimpedance-based biosensor for detection of E. Coli. **(a)** Photo of the sensor. **(b-g)** Fabrication steps of the microchip: **(b)** 100 nm thick Cr/Cu seed layer deposition, **(c)** Photoetching, **(d)** Coating of the bottom NiFe layer by electrodeposition, **(e)** Cu deposition, **(f)** Top NiFe layer formation, **(g)** Reactive ion etching to give the final shape. **(h)** Detection principle with Dynabeads, reprinted from Yang et al. (2015) with permission from Springer. **(C)** MEMs-based, optical waveguide grating system for biosensing of different analyts: **(a)** optical transducing setup. **(b)** and **(c)** shows the refractive index change following the adsorption of target molecules on the sensor surface lead to alteration in coupling angle (θ_c), which can be measured by a temporally shifted intensity signal I. While S shows substrate, F and C show waveguide film and cover layer.**(d-i)** Fabrication process of the sensing system including interference lithography **(d),** reactive ion etching **(e)**, sputtering of a Ta_2O_5 layer **(f)**, deposition of photoresist **(g)**, sputtering of a second Ta_2O_5 layer **(h)**, and lift-off of the additional Ta_2O_5**(i),** reprinted from Kehl et al. (2016) with permission from Elsevier.

Figure 6. Improvments in MEMs technology: **(A)** Schematic comparison of flexoelectric actuation and piezoelectric bimorph actuation, reprinted from Bhaskar et al. (2015) with permission from Nature Publishing Group. **(B)** System-on-chip based on polysilicon nanowire technology towards to detection of Hepatitis B Virus DNA and cardiac troponin I protein, reprinted from Huang et al. (2013) with permission from Royal Society of Chemistry.

Table Captions

Table 1. Sensing systems designed with applying various patterning techniques

Table 2. Some microfluidic biosensors: Design, description and performance

Highlights

- Micro/nano patterning techniques provid some opportunities for high throughput screen or analysis of biological molecules.
- Microfluidic technology helps to better simulate and quantificate the biologicals.
- Microelectromechanical systems based sensors are promising candidates as implantable sensors.

Table 1

Patterning Technique	Patterned Material	Pattern Shape	Detected Biomolecule	Detection Methode	Detection Limit	Literature
Soft Lithography	PS/PSMA	Micropillar	Anti-IgG	Fluorescence	0.03 $\mu g.mL^{-1}$	Lee et al., 2011
Stencil Lithography	Sillicon Nitride	Au squar nanodot	Streptavidin	SPR	100 nM	Vazquez-Mena et al., 2011
Wet Ethcing	ITO	Whell-like	Glucose, Cholin, Lactate	ECL	14, 40, 97 μM	Zhou et al., 2014
Nanoimprint Lithography	Glass	Au elliptical nano-disc	PSA	SPR	0.0012 $ng.mL^{-1}$	Lee S-W et al., 2011
Nanoimprint Lithography	PET	NanoDome	IgG	LSPR	3.4 nM	Endo et al., 2010
Nanoimprint Lithography	Photonic Crystal	Nanohole	Influenza Virus	Reflectometry	10 $pg.mL^{-1}$	Choi and Semancik, 2013
Soft Lithography	PEG Hydrogel	pH responsive-Circular	Streptavidin	Fluorescence	-	Lee et al., 2008
Non-contact Robotic Printer	APTES-coated glass slide	Spot	*Escherichia coli*	Fluorescence	25 bacteria each have 1 nl volume	Melamed et al., 2011

ECL: Electrochemiluminescence SPR: Surface Plasmon Resonance ITO: Indium-Tin Oxide PSA: Prostate Specific Antigen IgG: Immunoglobulin G PET: Polyethylene Terephthalate PEG: Polyethyleneglycole PS: Polystyrene PSMA: Poly(styrene-alt-maleic anhydride)

Table 2

Detection Method	Description	Target Molecule	Detection Limit	Literature
Chronoamperometry	Lab-on-a-CD system for whole blood analysis.	Glucose Lactate Uric acide	0.3 mM 0.1 mM 0.7 mM	Li et al., 2013
LSPR	Microchip having channels with 60 μm of depth and 400 μm of width.	Bovine somatotropin	185 pM	Sadabadi et al., 2013
Amperometry	Microfluidic chip printed with CNTs modified gold electrode having surface area of 410μm X 300μm.	Vasopressin	43 pM	He et al., 2013
Amperometry	Microchip having two parallel strips of 6.3 mm double-sided polyimide tape with Pt working electrode.	Nitric Oxide in whole blood	840 pM	Hunter et al., 2013
Colorimetric	2D stacking lateral flow immunoassay with a microfluidic network.	Dengue-specific immunoglobulins in salivary	20 $ng.mL^{-1}$	Zhang et al., 2015
SPR	Nanoplasmonic microfluidic transmission-based SPR biosensor consisting of a top control layer, a middle membrane layer an a nanostructured flow layer. The microchi has monolithically integrated blazed nanograting structures on the bottom.	CD44 antibody	10.53 pM	Malic et al., 2013
LSPR	The microchip was prepared by interference lithography and consists of gold nano-mushroom arrays.	Alpha-fetoprotein in serum	24 $ng.mL^{-1}$	Li et al., 2015
Amperometric	Microfluidic device was composed of three layers: Glass slide with micropatterned Au electrodes, PDMS layer with fluid channels and microcups, and another PDMS layer for controlling of microcups.	Transforming Growth Factor-β Releasing from Liver Cells	1 $ng.mL^{-1}$	Matharu et al., 2014

Optic detection using cell phone camera	A power-free and portable ELISA on chip platform consisting of 3 PDMS layer. The Chip platform has eliminated the need for pumps and valves through utilizing a simple permanent magnet and magnetic nanoparticles	PSA in serum	3.2 ng.mL^{-1}	Ahmed and Azzazy, 2013
Fluorescence	A microfluidic device generating and manipulating <10 fL droplets at rates of up to 1.3x10^{6} per second.	PSA in buffer	46 fM	Shim et al., 2013
Fluorescence	PDMS/paper/glass hybrid microfluidic system integrated with aptamer-functionalized graphene oxide GO nano-biosensors for simple, one-step, multiplexed pathogen detection.	Acidophilus Aureus Enterica	11 cfu.mL^{-1} 61 cfu.mL^{-1} 800 cfu.mL^{-1}	Zuo et al., 2013
DPV	Multiplex microfluidic chip for real-time quantitative differentiation of respiratory tract infections bacteria based upon loop-mediated isothermal amplification of nucleic acid.	Tuberculosis Influenza Pneumonia	28 copies/μL 17 copies/μL 16 copies/μL	Luo et al., 2014
Optical microscop	Microfabricated PDMS devices with Hele-Shaw shaped microchannel.	CD4+ lymphocytes from unprocessed, unlabeled whole blood	200 cell.μL^{-1}	Cheng et al., 2007
FRED	33-channel microfluidic chip integrated with the GO-based FRET aptasensor enabling to detect targe cells and also allowing a high-throughput assay of different cell samples simultaneously.	T-cell acute lymphoblastic leukemia cells	25 cell.μL^{-1}	Cao et al., 2012

LSPR: Localized Surface Plasmon Resonance, DPV: Differential Puls Voltammetry, FRED: Förster resonance energy transfer

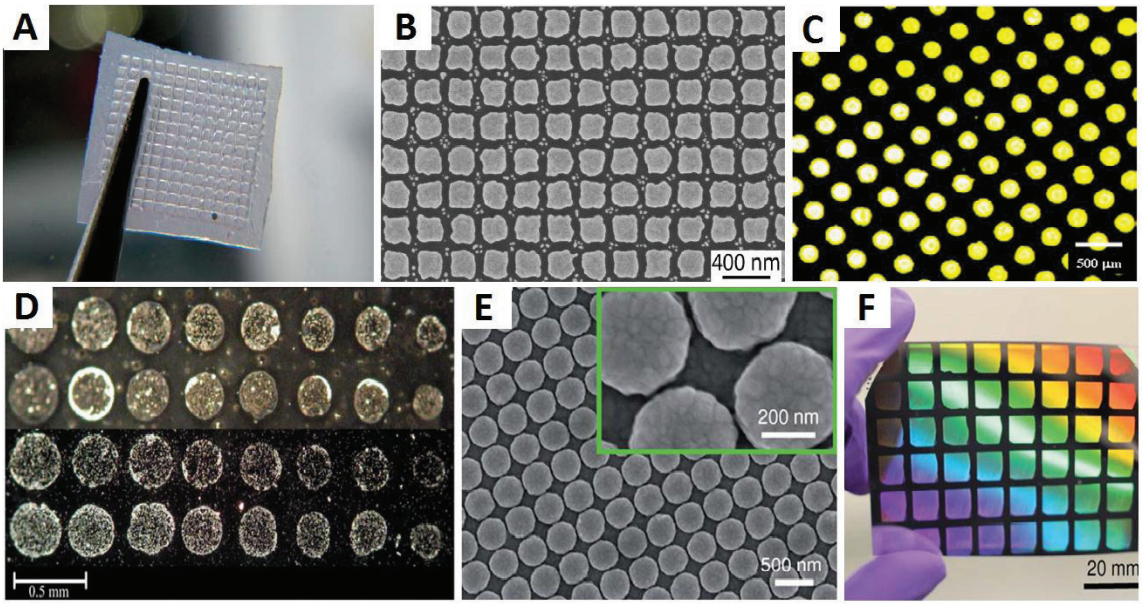


Figure 1

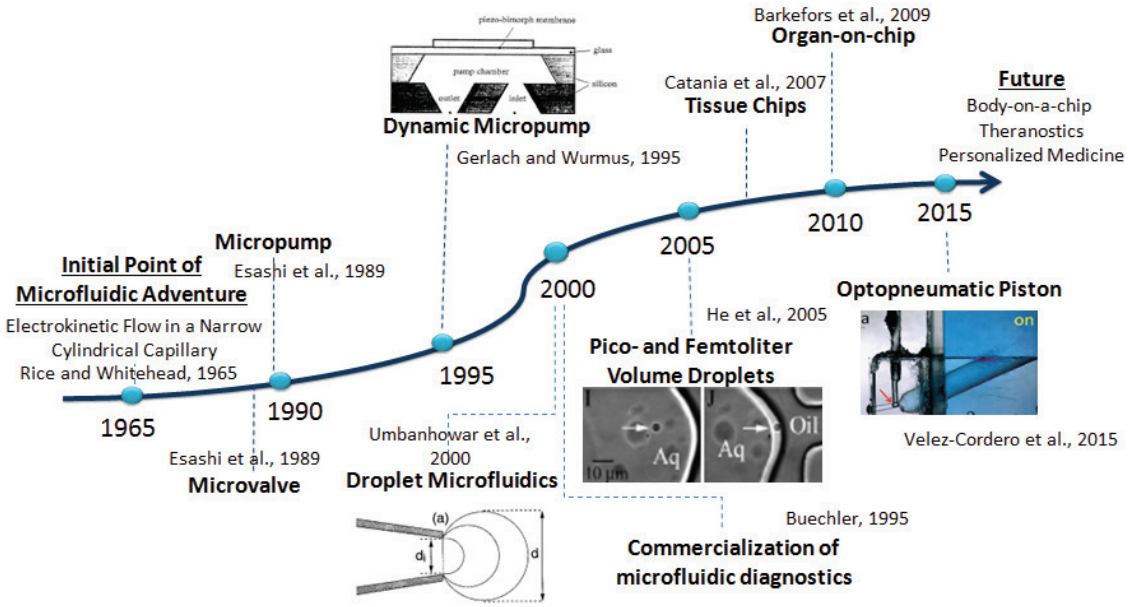
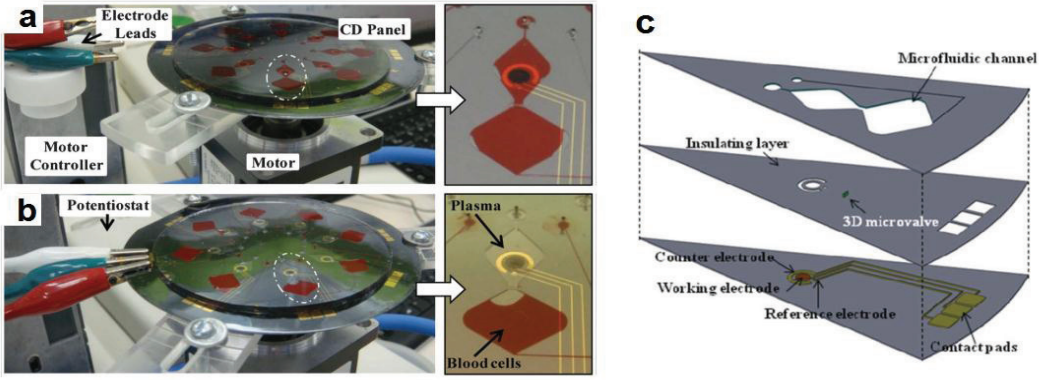
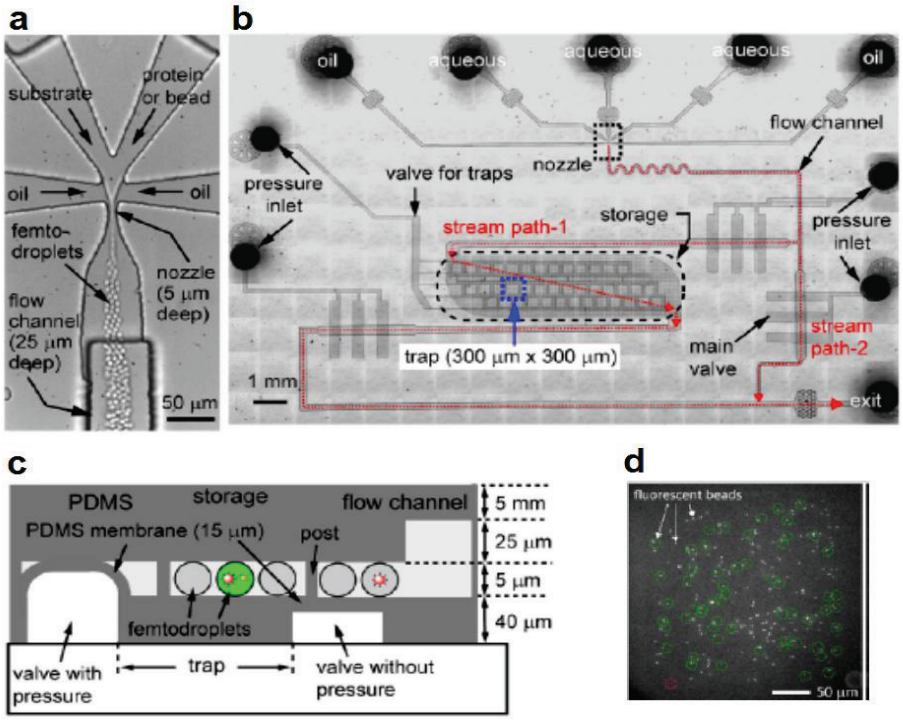


Figure 2

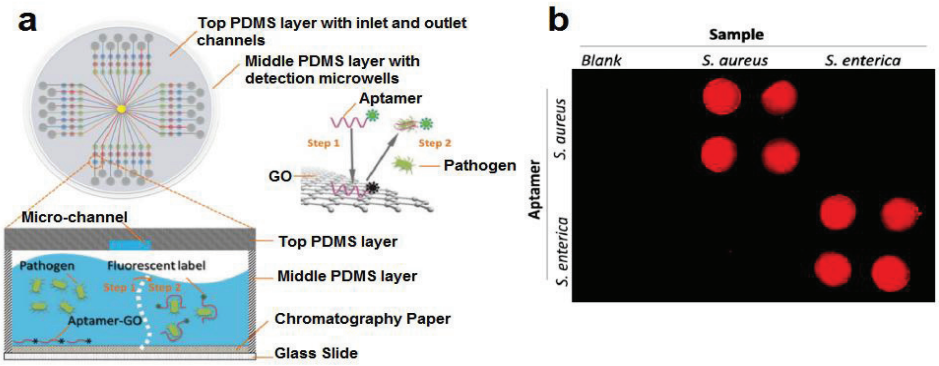
(A) Microfluidic Biosensor



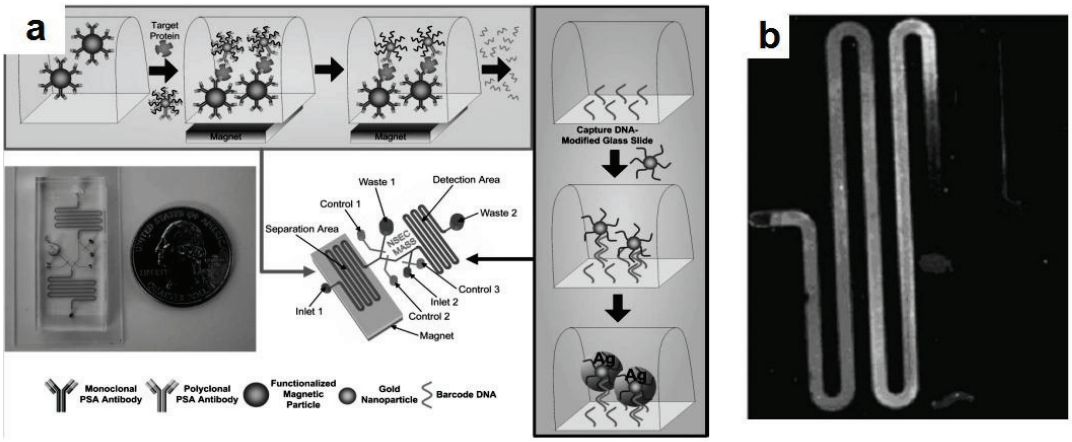
(B) Femtoliter Droplet Based Microfluidic Immunosensor



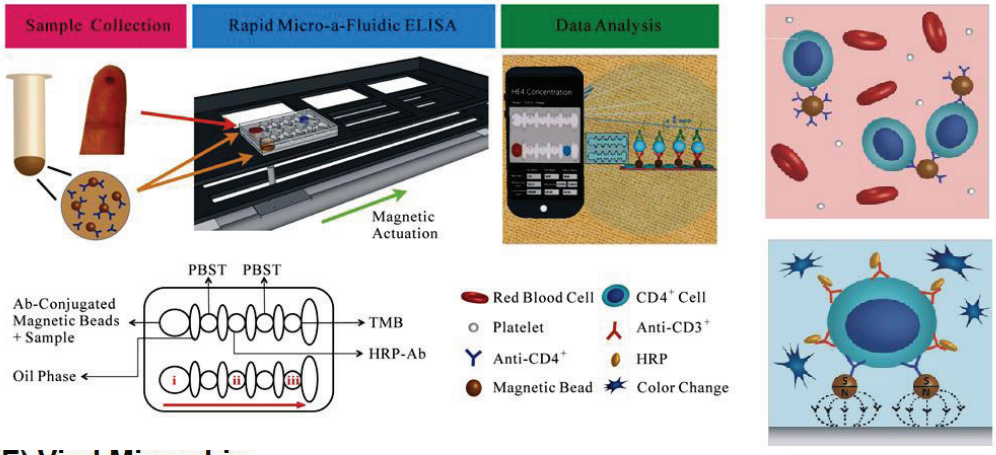
(C) Microfluidic Bacterial Sensor



(D) Microfluidic DNA Sensor



(E) Micro-a-ELISA Cell Chip



(F) Viral Microchip

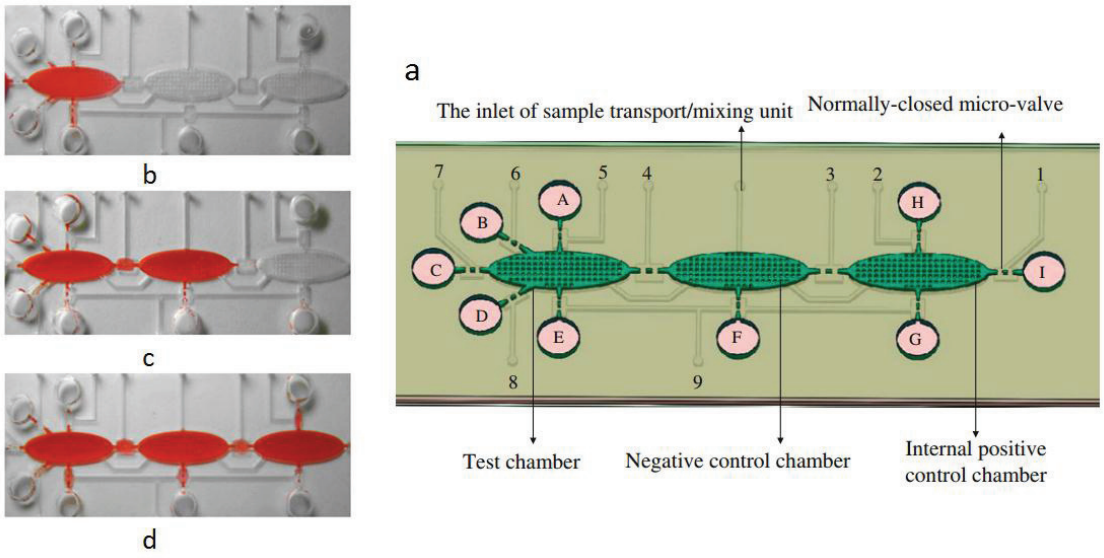


Figure 3

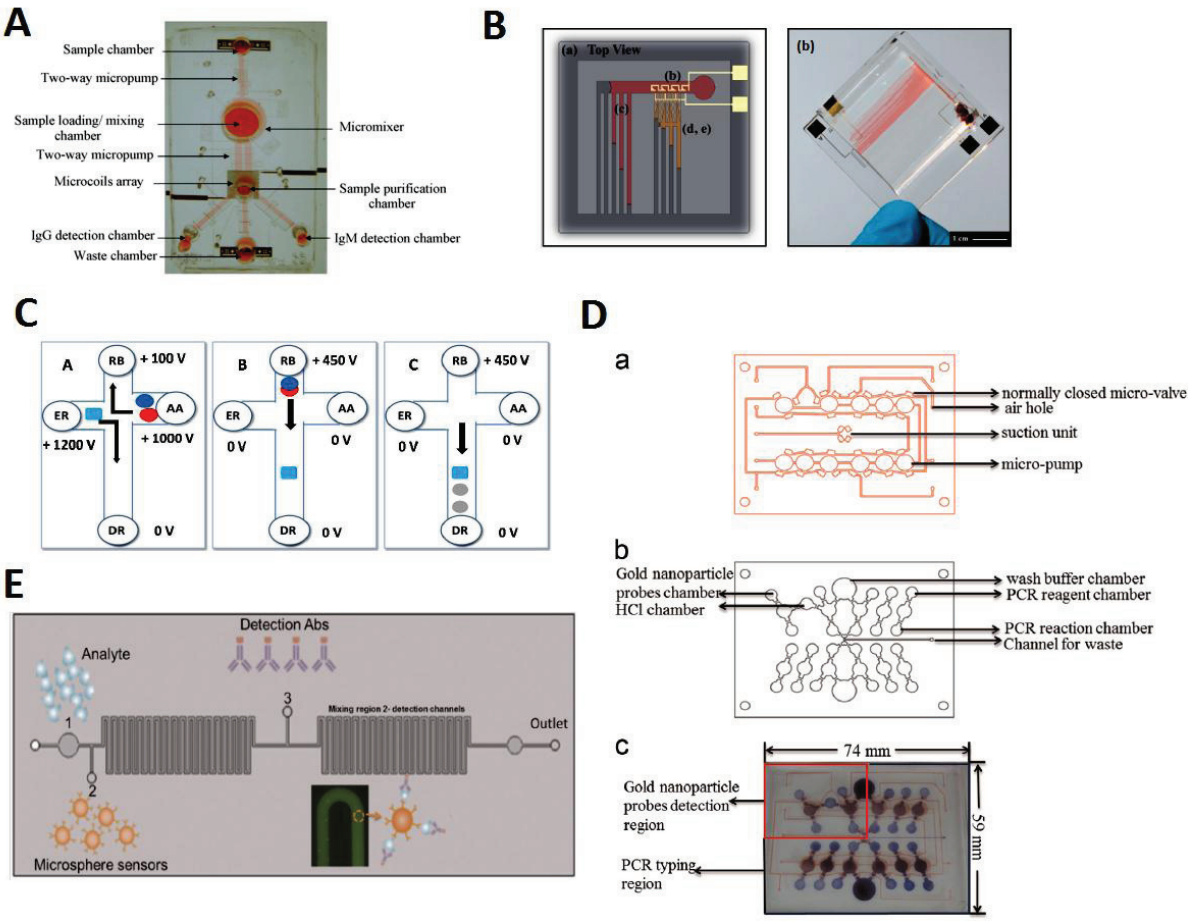


Figure 4

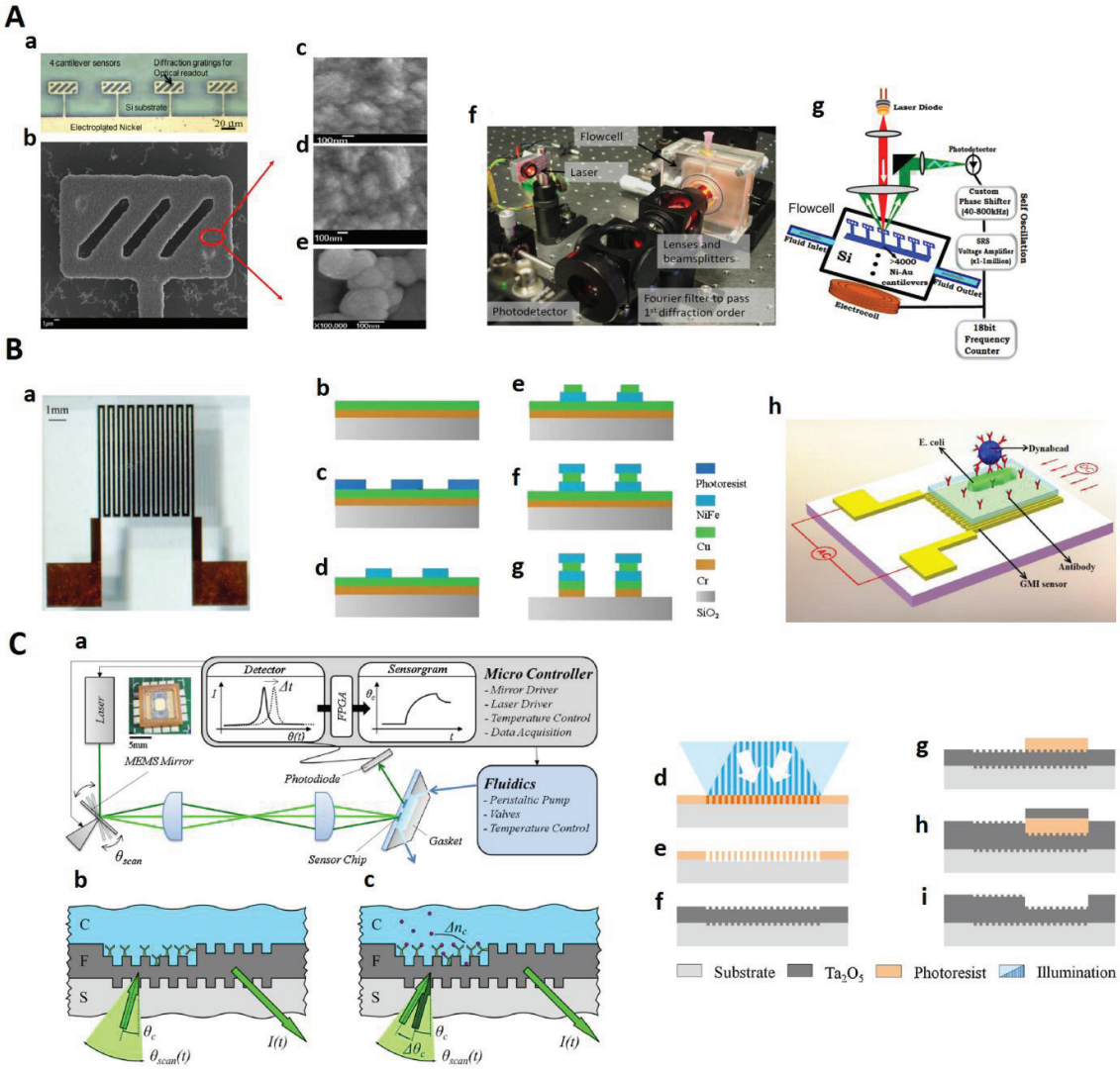
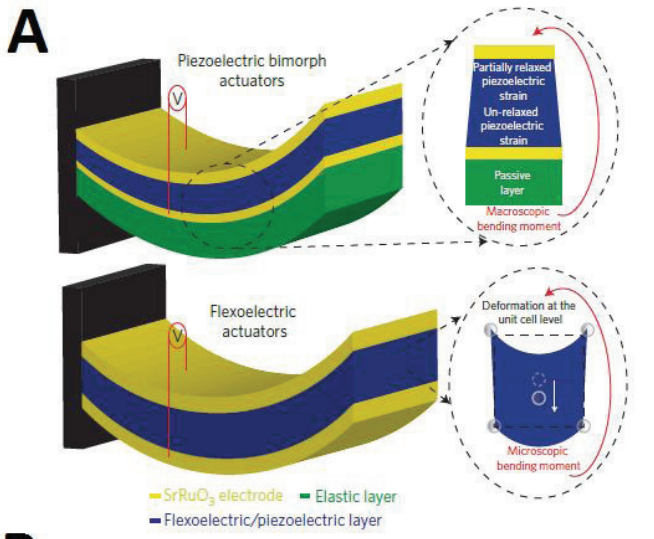


Figure 5



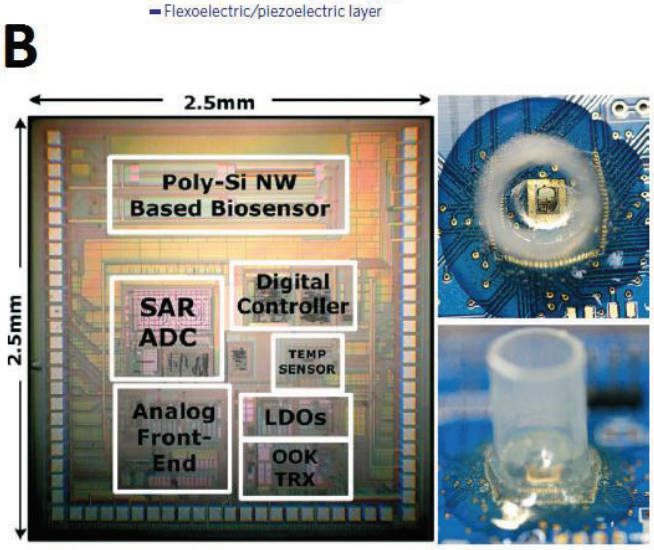


Figure 6