African Journal of Biotechnology Vol. 12(36), pp. 5486-5495, 4 September, 2013
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.3826
ISSN 1684-5315 ©2013 Academic Journals

Review

Nano lab-on-chip systems for biomedical and environmental monitoring

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Accepted 20 June, 2012

In recent years, nano lab-on-chip (NLOC) has emerged as a powerful tool for biosensing and an active area of research particularly in DNA genetic and genetic related investigations. Compared with conventional sensing techniques, distinctive advantages of using NLOC for biomedicine and other related area include ultra-high sensitivity, higher throughput, *in-situ* monitoring and lower cost. This review aims to summarize recent advancements in two major types of NLOC sensing approaches, label and labelled free NLOC, as well as their biomedical applications. The state-of-the-art on how these sensors interface with nano/microfluidics is then presented and the latest papers in the area summarized and also proposal to develop compact NLOC with four different sensing elements with two different mechanisms, common and separate padding is prospected.

Key words: Nano lab-on-chip, *in-situ*, nano/microfluidics, sensors, DNA.

INTRODUCTION

In recent time, the quest for biological systems from molecules, through cells, to small multicellular organisms has explosively grown based on the advancement in nanotechnology (Kuo-Kang et al., 2010). This enabling technology allows sensing of ever-decreasing sample volumes and target analyte concentrations in ways that are not possible using conventional testing systems. Such technology also has the benefit of scaling the dimensions that enables a range of fundamental features to accompany system miniaturization such as reduced reagent consumption, high temporal resolution due to rapid response, high throughput, enhanced analytical performance, less waste, low unit cost, reduced energy consumption, and reduced dimensions when compared to microscale techniques (Squires and Quake, 2005; Kuo-Kang et al., 2010). It is a powerful tool holding great promise to facilitate novel experiments with unprecedented performance and has already found unique applications in chemical and system biology (Bringer et al., 2004; Breslauer et al., 2006; Weibel and Whitesides 2006), high-throughput biological screening (Hong et al., 2009), cell analysis and clinical diagnostic (Kuo-Kang et al., 2010; Sato et al., 2008) as well as point-of-care (POC) ion analysis for biomedical and environmental monitoring (Gardeniers and Berg, 2004). The significant development of bioanalysis and clinical analysis has mainly been driven by the strong demand for fast and reliable results, which are essential for early diagnosis and further medical treatment. Results concerning potential drug targets, vaccine studies and speciation of toxic substances must also be of the highest reliability (Kuo-Kang et al., 2010). These bioanalytical challenges in many cases can be solved using specifically designed and fabricated miniaturized tools called lab-on-a-chip systems or micro total analysis systems (μTAS) (Harrison et al., 1993). Advances in technology have allowed chemical and biological processes to be integrated on a single platform. Adaptation of these approaches to lab-on-a-chip or μTAS formats is providing a new kind of research tools for the investigation of biochemistry and life processes.

Tremendous effort and promising experimental results were established in lab-on-chip (LOC) research, however, the fundamental mechanism of the sensing unit design and the fluidic delivery system for the platform of the in-

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interaction remain poorly understood (Anderson and Berg, 2004; Jungkyu et al., 2009). For the fabrication of sensing element and the probing unit, alignment and exposure are the most critical steps in the process, the resolution requirements and precise alignment are vital; each mask needs to be precisely aligned with original alignment mark. Otherwise, it cannot successfully transfer the original pattern to the water surface causing device and circuit failure (Hong, 2009; Hashim et al., 2008). Precise transfer of pattern means guarantee in high repeatability and reliability, high throughput and low cost of ownership (Hong et al., 2008; Bien et al., 2009). By improving this resolution and alignment precision, the minimum size can be further reduced to 1 nm and beyond and other important aspect of achieving minimum precised size is that the photo resist must be very sensitive to the exposure light to achieve reasonable throughput. However, if the sensitivity is too high, other photoresist characteristics can be affected, including the resolution (Hong, 2009; Hallstedt et al., 2008; Ra et al., 2008). In the proposed design, we have designed 3 masks for the fabrication of poly-silicon nanowire (SiNWs) during pattern transfer process steps; each was precisely aligned with the previous to achieve successful pattern transfer in the fabrication. There was a very little room for alignment error; we were able to achieved less than 2% error of the critical dimension and for the sensing unit to reach its potential, it must be integrated with sophisticated fluid delivery system for complete diagnostic system, therefore, there is a need for microfluidics chip to be integrated with the sensing to achieve an affordable and reliable lab-on-chip.

LAB-ON-CHIP SYSTEM AND COMPONENTS

Systems

LOC systems for biosensing normally consist of a set of sensing unit, fluidic operation and probing units which allow different biomolecules to be detected and assayed in an easy and flexible manner. Overall, the chip-based platform which has good integration with micro/nanofluidic components is capable of detection for biomolecules.

Sensing unit

Nanoscale sensors have critical structural dimensions of less than 100 nm. Nanoscale transduction mechanisms are typically classified into two categories: label-based and label-free. Label-based transduction mechanisms typically rely on the presence of an added labelling molecule or structure and LOC sensing elements have been used to elucidate a wide spectrum of genetic and proteomic information with improved selectivity and sensitivity when compared to traditional methods. For example, nanotechnology-based, single-cell detection methods have been used to investigate how a cell responds to environmental changes, interacts with neighbouring cells, and expresses specific genes as it responds.

Label-free sensing method

Label-free methods have emerged as a potential way to avoid possible structural and functional alterations of target molecules while providing acceptable sensitivity and selectivity. Assay protocol simplification, which facilitates portable or POC sensing, is an additional expected advantage. With recent advances in micro and nanotechnologies, label-free biosensors have achieved attogram (Jungkyu et al., 2009; Jinquan et al., 2009) sen-sitivity and tremendous high-throughput analysis capabilities. Here, we mentioned the current state-of-the-art in label-free detection techniques, including nanowire, surface plasmon resonance, surface- enhanced raman scattering, micro/nano-cantilevers, and nanopores but for the purposes of this review, nanowire is fully described

Nanowire: SiNWs biosensors along with nanotube (Kong et al., 2000) and conducting-polymer nano wire (Ramanathan et al., 2005) are promising label-free electronic biosensors (Figure 1). The most important and powerful advantage of SiNWs sensors is the possibility of multiplexed and real-time detection. The progress in nanofabrication techniques allows us to make an array of identical structures, which leads to massively parallel measurements. As most nanofabrication techniques originated in microelectronics, they can be easily scaled-up and transferred to a mass production line with high reliability. The underlying mechanism of nanowire sensors is based on the principle of field-effect transistors (FETs). Figure 1a shows a p-type SiNWs FET and its response to different gate voltages. In the case of a p-type semiconductor, a positive gate voltage depletes carriers and reduces the conductance, while a negative gate voltage leads to an accumulation of carriers and an increase in the conductance. For biosensors, binding of a charged species on the surface of the SiNWs is analogous to applying a gate voltage. By monitoring the conductance change, the binding of targets to probe molecules can be detected on the Si surface. Several research groups have already demonstrated the successful solution-phase SiNWs sensing of DNA viruses (Patolsky et al., 2004), small molecules (Wang et al., 2005) and proteins (Stern et al., 2007a; Zheng et al., 2005). The current detection sensitivity of fibromyalgia (fM) range is, in several orders of magnitude, more sensitive than a conventional enzyme-linked immunosorbent assay (ELISA) assay. For most of those experiments, however, low salt buffer solutions were used to avoid the screening effect associated with solution counter ions (Stern et al., 2007b). The charge of target molecules is screened by the counter ions in solution and effective only on the scale of the Debye length.

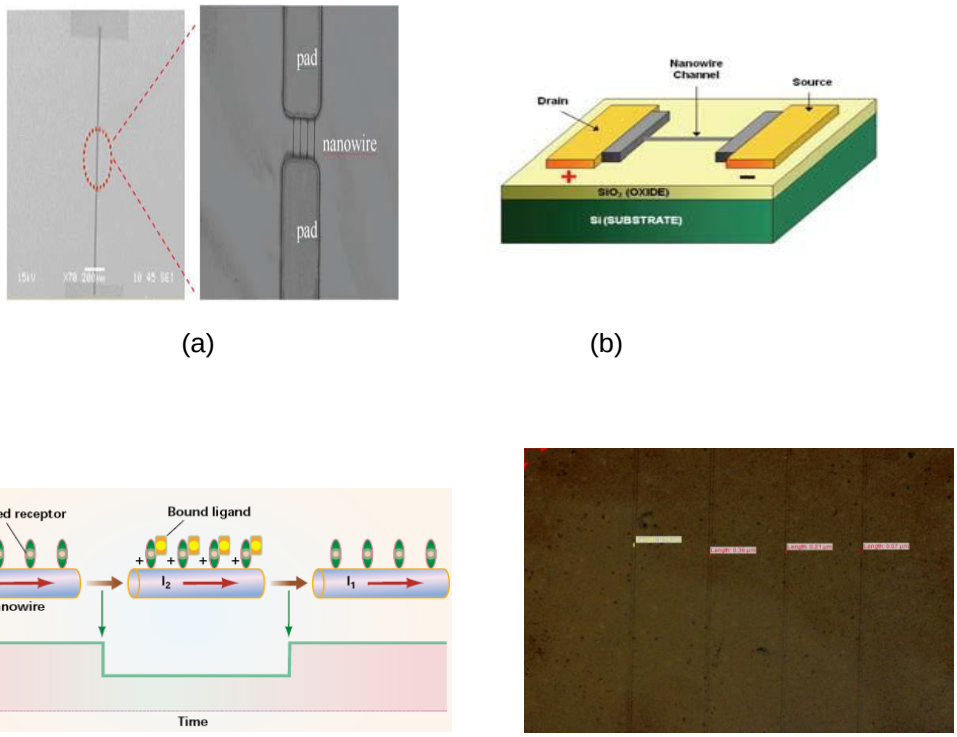

Figure 1. (a) Schematic of a regular p-type ET device. S and D correspond to source and drain; (b), HPM image observed the connected nanowire between the two electrode pads and the 3-D model of overall pattern fabricated on the sample; (c), operation principle of nanowire filled with DNA (Uda et al., 2008); (d), the SEM image of the silicon nanowire (Jungkyu et al., 2009).

Several research groups recently focused on the importance of the Debye length in this application (Stern et al., 2007b; Zhang et al., 2008). The Debye length for a 0.1 M solution is about 1 nm and biologically relevant media is typically a 0.14 M electrolyte. Since the salt concentration and pH are important factors for the binding between biomolecules, it is necessary to find an alternative way of overcoming the charge screening to perform an ideal sensing measure-ment with NW-FETs (Huan-Xiang, 2001). For DNA sensing, the charge screening can be overcomed by electro statically immobilizing ssDNA on the SiNWs surface (Bunimovich et al., 2006). With antibodies, however, the bimolecular recognition event occurs at 10 nm away from the wire due to the antibody's large size. In biological media, the binding event usually takes place farther away than the Debye screening length; so alternative small capture agents are required to bring the binding event closer to the nanowire (Figure 1c). Thus, finding small molecules that have the same specificity for proteins as antibodies and that can distinguish between slightly different proteins is critical. Due to their small scale, high sensitivity and real-time detection capability, nanowire based sensors could be used to study single cells (Figure 1d).

Label-based sensing method: Biomolecules such as proteins and nucleic acids often cannot be recognized directly due to their small size. To track these biomolecules and their activity, probes for these target molecules or the target biomolecules themselves can be labelled by conjugation with a detectable agent, commonly a fluorophore or an enzyme. Labelling methods allow high sensitivity and these approaches are developed to the point that they give reproducible results. These agents for labelling proteins, nucleic acids, and other molecular probes are called tags. These tags have unique detectable properties such as radioactivity, chromogenicity, fluorescence, or magnetism. Additionally, electrical and electrochemical principles, based on the properties of labelled probes have been developed to establish corresponding detection methods through a target binding technique (Figure 2). Having a uniquely detectable property, most tags can be functionalized to link to a specific molecular probe. In a related approach, instead of having a detectable group directly attached to a probe molecule, a recognition reagent having strong affinity for a secondary probe can be used to detect the target molecules. A variety of interaction pairs, such as biotin-avidin, hapten-antibody, and DNA–RNA hybrids, etc., are already in use (Kessler et al., 1992; Jungkyu et al., 2009). This two-stage detection scheme can be utilized when a primary-labelled probe is not available (Jungkyu et al., 2009). A schematic diagram for both labelling approaches is shown in Figure 2a. In the past, scientists mostly relied

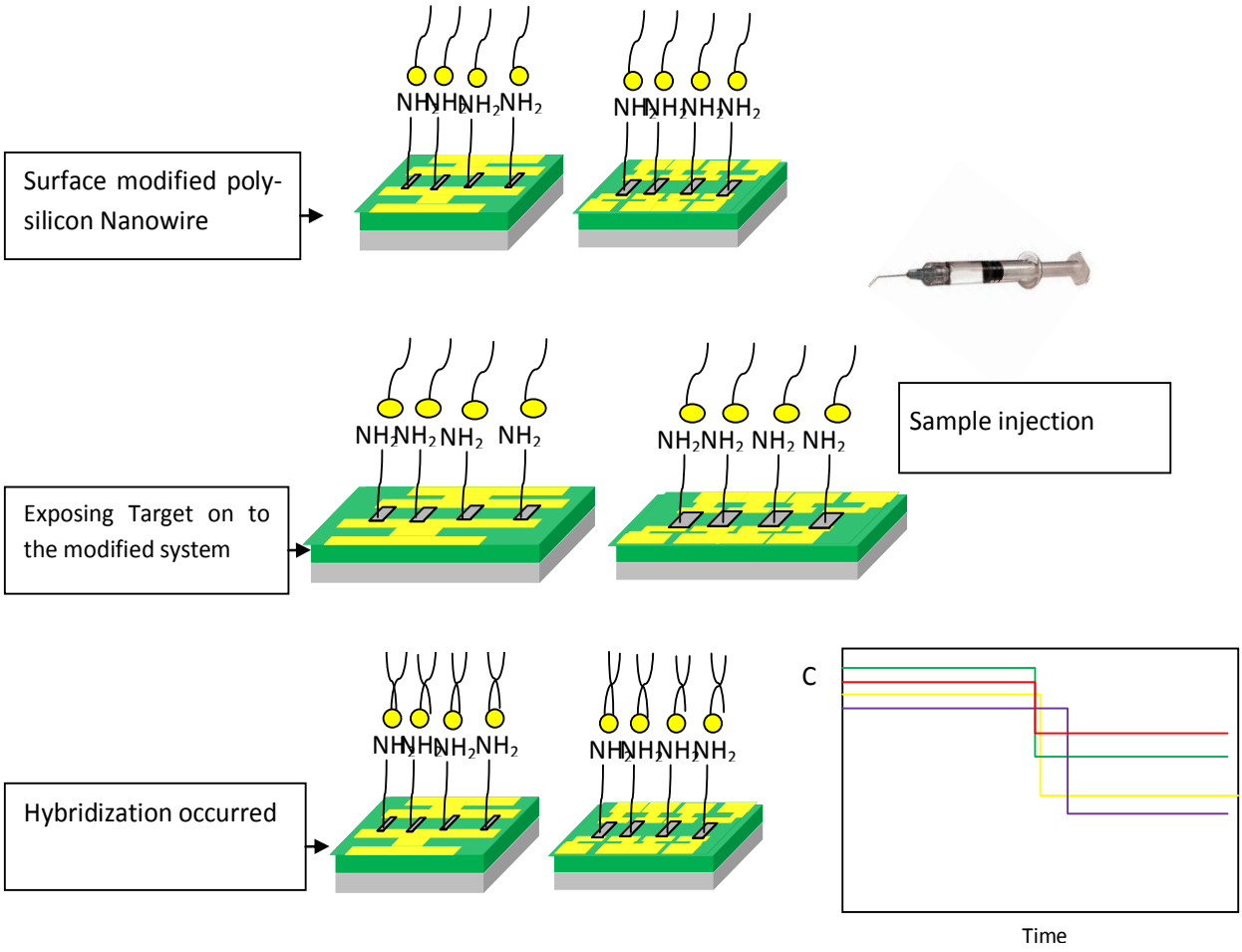

Figure 2. (A) Schematic diagram of two approaches to label-based biomolecules detection: (a), Direct method; (b), indirect method; (B), schematic diagram of nano-shell (quantum dot) detection by attaching probe molecules to the nanoparticles surface; (C), schematic diagram of using a gold nanoparticles as a label and possible detection methods (Jungkyu et al., 2009).

on radioactive probes to detect samples. However, safety and convenience concerns spurred the development of alternative techniques (Gary, 1993). Among a range of options, current bio-molecules detection methods have mainly employed fluorescent labels, quantum dots, or heavy atom complex nano-particles labels. Chromogenic labels are also available, but they have been replaced with fluorescent labels which give larger quantum emission yield upon excitation resulting in better delectability (Jungkyu et al., 2009). Bioluminescence, which generates detectable light as a result of biochemical reactions, is another popular technique for bio detection. Other detection methods are Au nanoparticles and magnetic nanoparticles labels. These techniques are emerging as better substitutes in terms of simplicity, sensitivity, specificity and reliability for the current standards.

Fluid delivery unit

Microfluidic systems for biosensing normally consist of a set of fluidic operation units that allow different biomolecules to be detected; the chip is capable of sampling, filtration, pre-concentration, separation, restacking, and detection for biomolecules and based on their flow type, microfluidic system can be categorized into two main types, continuous and discrete. Continuous-flow microfluidic operation is a promising approach because it is easy to implement and less sensitive to protein fouling problems. Continuous-flow devices are adequate for many well-defined and simple biochemical applications, and for certain tasks such as chemical separation, but they are less suitable for tasks requiring a high degree of flexibility or complicated fluid manipulations. These closed-channel systems are inherently difficult to integrate and scale because the parameters that govern the flow field vary along the flow path making the fluid flow at any one location dependent on the properties of the entire system. Permanently-etched microstructures also lead to limited reconfigurability and poor fault tolerance capability. In other words, droplet-based microfluidic systems are currently an emerging area of microfluidic research. One of the most popular means is to inject multiple laminar

streams of aqueous reagents into an immiscible carrier fluid and therefore to induce flow instability instantly for forming the droplets (Tice et al., 2003). There are several distinctive advantages based on droplet-based microfluidic systems. First, the systems promise a new high-throughput technology that enables the generation of micro droplets in excess of several thousand per seconds (Huener et al., 2007). In addition, parallel and serial *in vitro* compartmentalization is possible with this technology. The reagents are con-fined inside the droplets in water-in-oil (w/o) emulsions and reagent transport occurs with no dispersion (Kelly et al., 2007). The fluidics components consist of micropump, valve, micromixer, separator and concentrator. Among these components, micropumps and micromixer are the key components for microfluidic applications due to their actively functioning capability. Therefore, for the purpose of this review, the micropumps and micromixer are fully described.

Micropump

Controlling fluid flow is crucial in microfluidic devices, especially for processing biochemical reactions. Such a process generally relies on active control by mechanical pressure (Linder et al., 2005; Marmottant and Hilgenfeldt, 2004), electroosmotic force, electro wetting (Jun and Kim, 1998); Huh et al., 2003) and electrochemical reaction (Gallardo et al., 1999). These active manipulations enable close control in a rapid and precise manner. Electrokinetic sampling has been widely used for microfluidic chip, especially for microfluidic chip electro-phoresis, because the electric field can be easily and precisely applied to the reservoirs on the chip. The popular mechanism used for these active micropumps is electrokinetic force. Based on the mechanism, various micropumps such as dielectrophoresis, asymmetric electric field, electroosmosis and electrophoresis (the latter two are considered as part of the electrohydrodynamic (EHD) phenomena) (Green et al., 2000; Brown et al., 2000) have been developed. Moving sample fluids and reagents on a biosensing microfluidic device requires developing a pressure difference in the flow path to direct fluid in one direction or another. Miniaturized versions of positive-displacement pump designs such as gear or peristaltic pumps have been proposed for microfluidic applications, but these all require some external power source or repetitive motion to control. It is desirable for fluidic motion in a passive microfluidic system design to be driven by a readily available force such as gravity, capillary action, absorption in porous materials, chemically induced pressures or vacuums (for example, by a reaction of water with a drying agent), or by vacuum and pressure generated by simple manual action. Wicking and capillary action have been widely used to motivate fluids for POC diagnostics. For example, low cost lateral flow tests demonstrate the elegant and inexpensive use of wicking to drive multiple sample types through all steps of an assay. One of the simple methods for transporting fluids on microfluidic devices is to apply pressure manually to deflect a diaphragm (Moorthy et al., 2004). Diaphragm membrane pumps have been demonstrated successfully in moving fluid on a microfluidic device. However, it is not easy to control the flow rate in a reproducible way. Zhu et al. (2002) reported a gravity microfluidic pump for producing constant flow rate. This passive system employs a microchannel and a gravity-driven pump consisting of horizontally oriented reservoirs that supply fluid to the microchannel at a substantially constant rate. The passive device may be useful for numerous microfluidic applications such as cell-size sorting (Huh et al., 2002). The pumps have been developed based on osmotic pressure as the actuation mechanism have been used in many drug-delivery applications to deliver medication over a prolonged period of time (Su et al., 2002; Su and Lin, 2004). The advantages of these pumps include simple construction and the absence of moving parts. Another passive system involves controlled evaporation of a liquid into a chamber with an absorption agent flow (Effenhauser et al., 2002). As fluid evaporates from the channel, capillary forces induce fluid flowing from reservoir to replace the evaporating fluid. This micropump has advantages of low cost, high reliability and constant flow rate over a long period of time. The major disadvantage of the evaporation micropump is the need to control environmental conditions for constant flow rates and lower flow rates. The micropumps have also been developed by employing fluid-responsive polymers to deliver fluids (Eddington and Beebe, 2004). Fluid-responsive polymers swell when exposed to certain environmental conditions, such as changes in moisture, pH or temperature. One recent fluid-responsive pump consists of an array of responsive polymers that deforms a flexible membrane made from polydimethyl siloxane (PDMS) and produces flow rates (Eddington and Beebe, 2004). The disadvantage of the pump is the requirement of pressure to inject the buffer solution in order to active the pump.

Micromixer

Mixing is a physical process to achieve homogeneity of the different components involved in certain process. In some cases, the mixing will be the rate determining step when the mixing time is in the same order or longer than the molecular reaction time. Because the fluid streams mainly appear naturally as laminar flow on a chip, the mixing will mainly depend on molecule diffusion. Mixing small amounts of reagents and samples in microfluidic channels or structures is a challenging task. Likewise, mixing in passive micromixer relies mainly on molecular diffusion and chaotic advection. To speed mixing process, the T-mixer or Y-mixer which consists of the inlets converging into a long microchannel has been developed as a simple and effective solution (Kamholz et al., 1999; Wang et al., 2005; Kamholz and Yager, 2003; Ismagilov et al., 2000). Other methods for fast mixing have been implemented through reducing the mixing path in a narrow

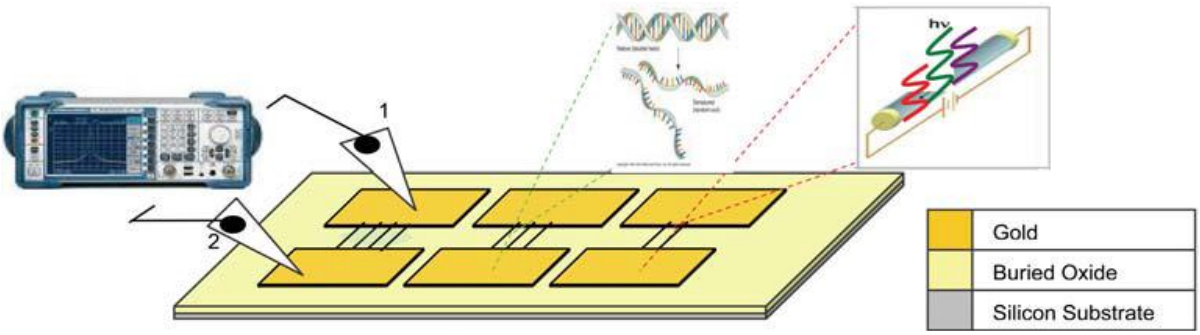

Figure 3. After gold pad formation, measurement of different nanowire pattern using spectrum analyzer semiconductor parameter analyzer are taken (Uda et al., 2008).

mixing channel (Veenstra et al., 1999) and realizing parallel lamination with multiple streams (Jackman et al., 2001; Koch et al., 1999). Besides diffusion, advection is another important form of mass transfer in flows with a low Reynolds number. However, advection is often parallel to the main flow direction, and is not useful for the transversal mixing process. The chaotic advection generated by special geometries in the mixing channel can improve mixing significantly. The basic idea is the modification of the channel shape for splitting, stretching, folding and breaking of the flow. The simplest method to get chaotic advection is to insert obstacles or structures in the mixing channel. However, it has been shown that eddies or recirculation cannot be generated in a microchannel, because of its low Reynolds number (Wang et al., 2002). The effective method to produce chaotic advection is to modify the wall of mixing channel with ribs, grooves and staggered-herringbone grooves. Johnson et al. (2002) were the first to investigate this phenomenon. They ablated the grooves on the bottom wall of the channel by laser. This structure allows mixing at a relatively slow velocity of 300 μm/s. Stroock et al. (2002) investigated two different groove patterns, slanted groove and staggered. The so-called staggered herringbone mixer can work well at low Reynolds number

Probing unit

Prior characterization and electrical testing, a process called metallization is done; a variety of conductors is applied in chip fabrication. Metal with high conductivity are widely used for interconnections forming microelectronics circuit. Metal such as copper, aluminium and gold are good conductors and are widely used to make conducting lines to transport signal; the requirements for metallization are low resistivity for low power consumption and quick device response, smooth surface for high resolution patterning process, high resistance to electro migration to achieve high device reliability, and low film stress for good adhesion to underlying substrate and other requirement are stable mechanical and electrical properties during subsequent processing, good corrosion resistance and relative receptivity to deposit and etch.

It is important to reduce the resistance of the inter-connection lines, since the chip speed/response is closely related to the RC time, which is proportional to the resistivity of the conductor used to form the metal line. The lower the resistivity, the shorter the RC time and the faster the chip. For the purpose of the review, presented here is focus on gold. A report from Uda et al. (2008) stated that a contact point is formed by deposition of aurum (gold) material prior to the fabricated nanowire. Gold is used to have a good reliability via contact and it has a very good conductivity. This is to ensure that the device has a good electron flow and no bias effect to the sensing nanowire. A layer of 500 nm thick of aurum is deposited using E-beam evaporator onto the surface of the fabricated nanowire. The layer is then coated and patterned using photolithography process to form the contact point. *Aqua Regia* is used for etching. Finally, the photoresist layer is removed to expose the gold pad for contact (Figure 3).

Prospect and the summary of the work

The SiNWs based biosensor fabrication process comprises of three major steps namely wire formation, wire trimming and gold pad formation. In development of nano lab-on-chip (NLOC), the sensing unit is very crucial and here we presented some critical parameters to be considered during fabrication of nanowire for biosensing, thus, alignment and exposure are the most critical steps in photolithography process, the resolution requirements and precise alignment are vital; each mask needs to be precisely aligned with original alignment mark. Otherwise, it cannot successfully transfer the original pattern to the wafer surface causing device and circuit failure. Precise transfer of pattern transfer means guarantee in high repeatability and reliability, high throughput and low cost of ownership. By improving this resolution and alignment precision, the minimum size can be further reduced to 1 nm and beyond and other important aspect of achieving

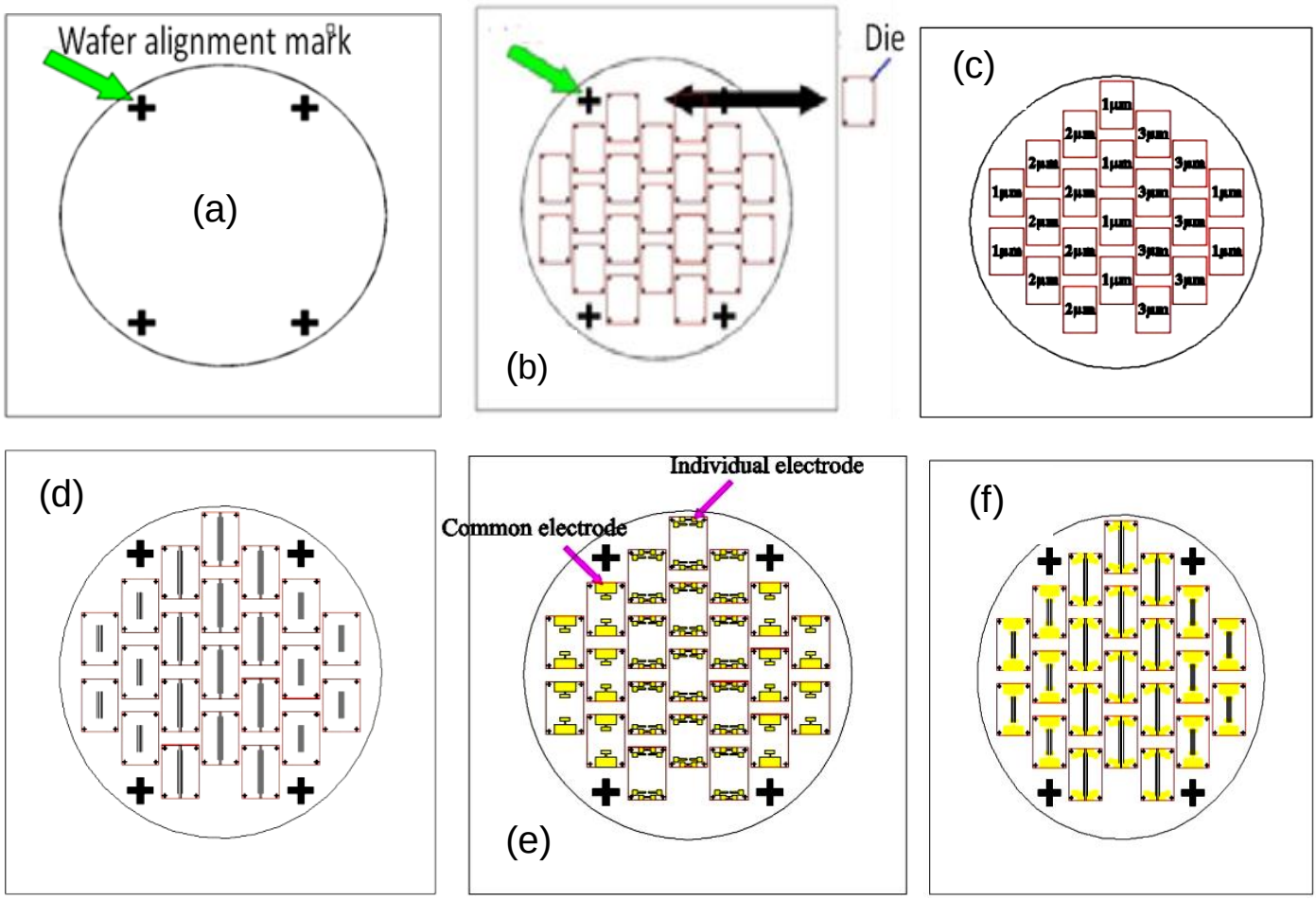

Figure 4. Mask design layouts. (a), Wafer alignment mark; (b), die alignment mark; (c), die position based on wire dimensions: 1, 2 and 3 µm; (d), wire mask layout; (e), common and individual electrode mask layout; (f), complete mask layout design.

minimum precised size is, the photoresist must be very sensitive to the exposure light to achieve reasonable resolution. In this study, we have 15 pattern transfer process steps to fabricate nanowire; each was precisely aligned with the previous to achieve successful pattern transfer in our design. There was a very little room for alignment error; we were able to achieved error free design to the critical dimension shown in Figures 4, 5 and 6

After poly-SiNWs, the fabrication process was completed, and then the fluidic chamber fabrication follows. This also consists of two major steps; started with the master template development and followed by replica fabrication of chamber and inlet/outlet channel. Subsequently, the inspection and characterization of the fabricated devices were conducted using transmission electron microscope (TEM), focused ion beam (FIB), field emission scanning electron microscope (FESEM) and atomic force microscope (AFM) instruments. After the testing of individual device was accomplished, the integration of the fabricated devices was done using plasma oxidation process with the two major integration steps throughput. However, if the sensitivity is too high, other photoresist characteristics can be affected, including the namely surface treatment and device mounting (Figure 7). Finally, SiNWs surface modification and DNA immobilization for DNA hybridization was done on SiNWs LOC for the testing and validation through electrical testing by using real biological samples. The integrated NLOC design with two probing approaches, in the common gold pad probing, the probe respond to any of the four transducer response and for the individual probing. As shown in Figures 7a and b, it has four different wires connected to the individual transducer and it means that, it responds individually for changes it experience from nanowire.

ACKNOWLEDGEMENT

The authors wish to thank Universiti Malaysia Perlis (UniMAP), Ministry of Science, Technology & Innovation (MOSTI) and Ministry of Higher Education for giving the opportunities to do this research in the Micro and Nano Fabrication Lab for providing us facilities to carry out this

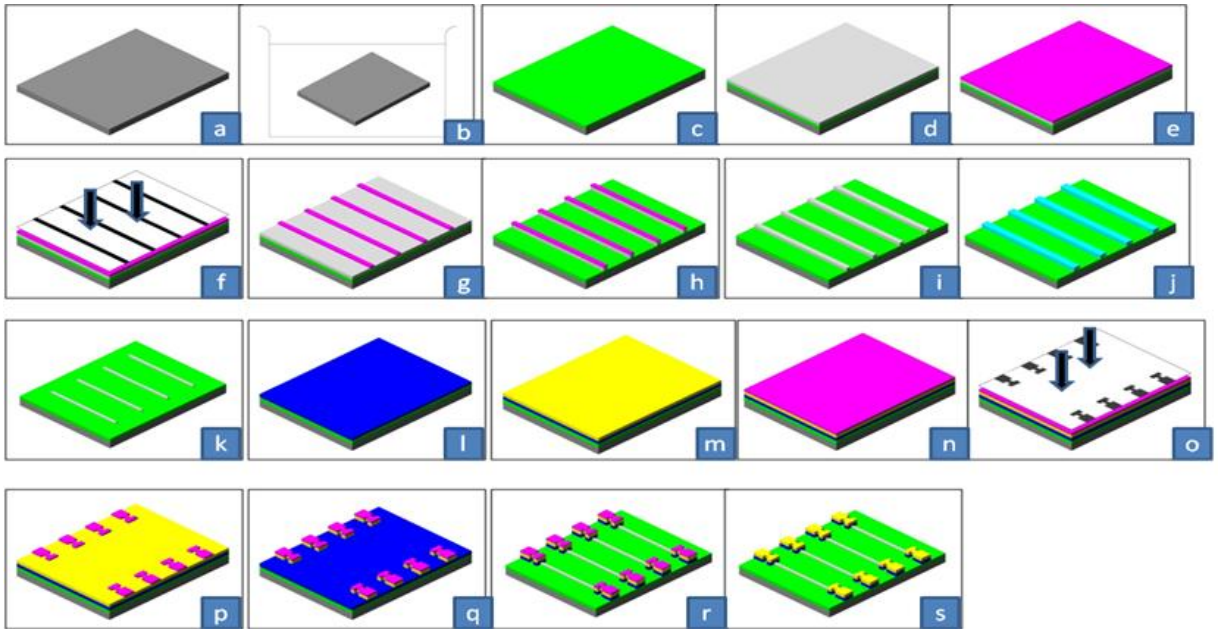

Figure 5. Fabrication process flow of polysilicon nanowire with common electrode. (a), Silicon wafer; (b), wafer preparation; (c), wafer insulation (Si_3N_4); (d), polysilicon deposition; (e), photoresist coating; (f), alignment and exposure; (g), resist development; (h), resist striping; (i), polysilicon microwire; (j), trimming process by plasma oxidation; (k), polysilicon nanowire; (i), titanium (Ti) deposition for metallic connection; (m), gold deposition for contact formation; (n), photoresist coating; (o), alignment and exposure; (p), resist development; (q), gold etching; (r), resist stripping; (s), Individual electrode polysilicon nanowire.

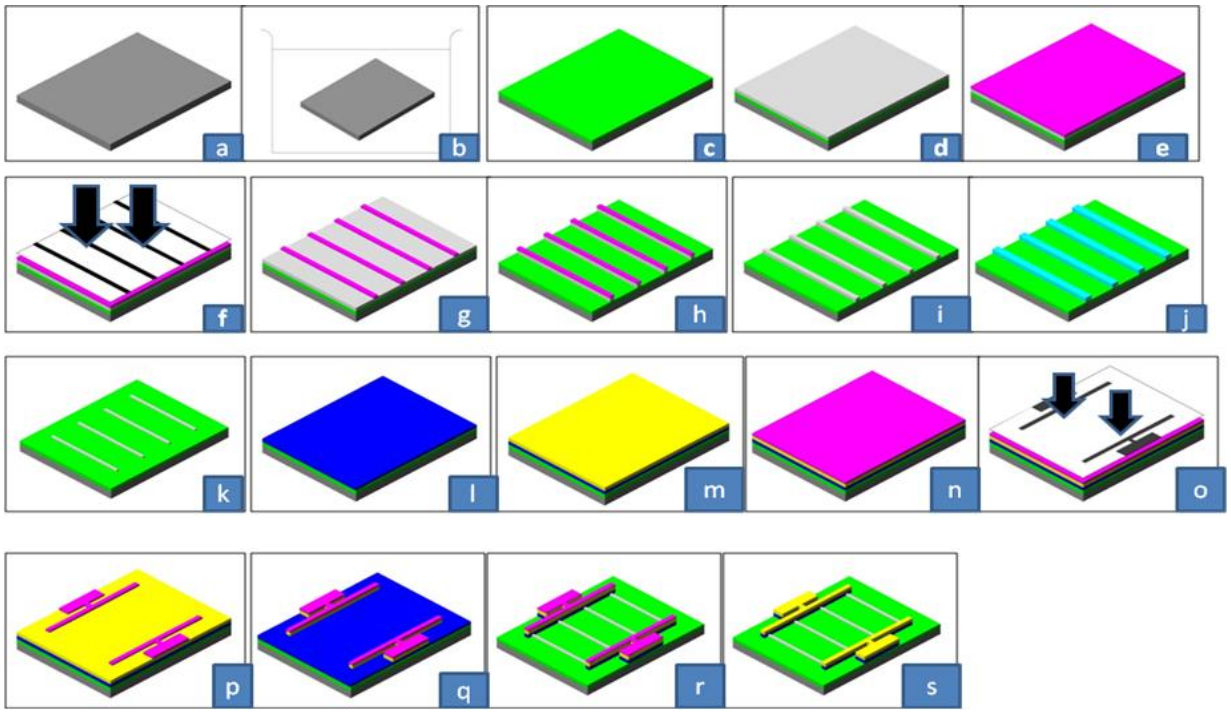

Figure 6. Fabrication process flow of polysilicon nanowire with common electrode. (a), Silicon wafer; (b), wafer preparation; (c), wafer insulation (Si_3N_4); (d), polysilicon deposition; (e), Photoresist coating; (f), alignment and exposure; (g), resist development; (h), resist striping; (i), polysilicon microwire; (j), trimming process by plasma oxidation; (k), polysilicon nanowire; (i), titanium (Ti) deposition for metallic connection; (m), gold deposition for contact formation; (n), photoresist coating; (o), alignment and exposure; (p), resist development; (q), gold etching; (r), resist stripping; (s), Individual electrode polysilicon nanowire.

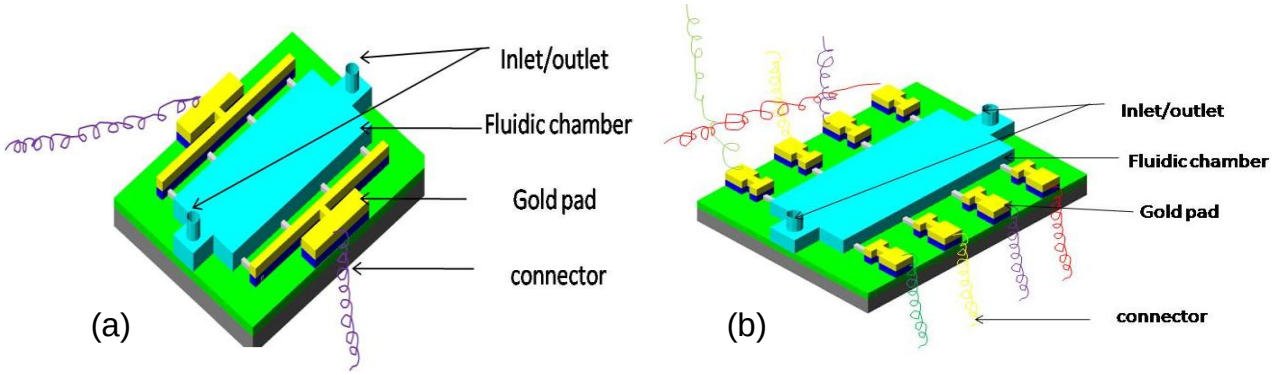

Figure 7. Integrated nano lab-on-chip. (a), Common gold pad probe device; (b), individual gold pad probe.

research. The appreciation also goes to all the team members in the Institute of Nanoelectronic Engineering especially in the Nano Biochip Research Group.

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