

Chapter 1

Design and Preparation of Microfluidics Device

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Abstract A rapid development has been witnessed since the birth of this miniaturized analytical equipment about forty years ago. And microfluidics, a discipline studying the manipulation and analysis of micro volume sample is also attracting the attention of academics with the annually increasing publications. At present, microfluidic device has become a powerful platform with diverse functions implicated in the fields of cellular biology, environmental study, food safety monitoring and micro synthesis, and has crossed the barrier of academic study into the daily life of normal customers. In this chapter, we will focus on several aspects concerning the design and fabrication of microfluidic devices, such as the development of device material, different methods of chip fabrication and functional units to realize purpose of intention. And in the end of this part, a brief future perspective is given to evaluate the potential applications of microfluidic device, especially with other portal devices like smart phone.

Keywords Microfluidics design · Chip fabrication · Functional units integration

1.1 Introduction

Microfluidic devices are termed as micro total analysis system (μ TAS) or laboratory on a chip (LOC), with the first reported work dating back to 1975. A gas chromatography at micro scale was reported then [1]. However, the academics did not respond actively to the new-born of this epoch-marking device firstly, but rather turned indifference to it with no follow up studies in a long term. Not until 1990s did the microfluidics device start to receive broad attention. The rapid development of integrated circuit processing technique and computer industrial, along with the urgent demand of bio-molecules separation devices redirected analytical devices to

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J.-M. Lin (ed.), *Cell Analysis on Microfluidics*, Integrated Analytical Systems,

https://doi.org/10.1007/978-981-10-5394-8_1

a path of miniaturization. Manz and coworkers designed an open-channel liquid chromatography based on microfabricated silicon chip in 1990 [2]. It is noteworthy that Manz is also the proposer of μ TAS concept [3]. In this work, the authors displayed integrated functions of sample pretreatment, separation and detection on one chip. In the last 20 years, researches concerning novel microfabrication strategies [4], development of new chip material [5] and application of microchip to detection [6, 7] have been increasingly studied and published. And microfluidic chip has become a multi-functional platform for various targets analysis (Fig. 1.1a) [8] and a powerful tool for biomedical applications (Fig. 1.1b) [9].

The advantages of applying microfluidic devices to analytical testing include: less consumption of reagents and samples, rapid reaction in microscale chamber,

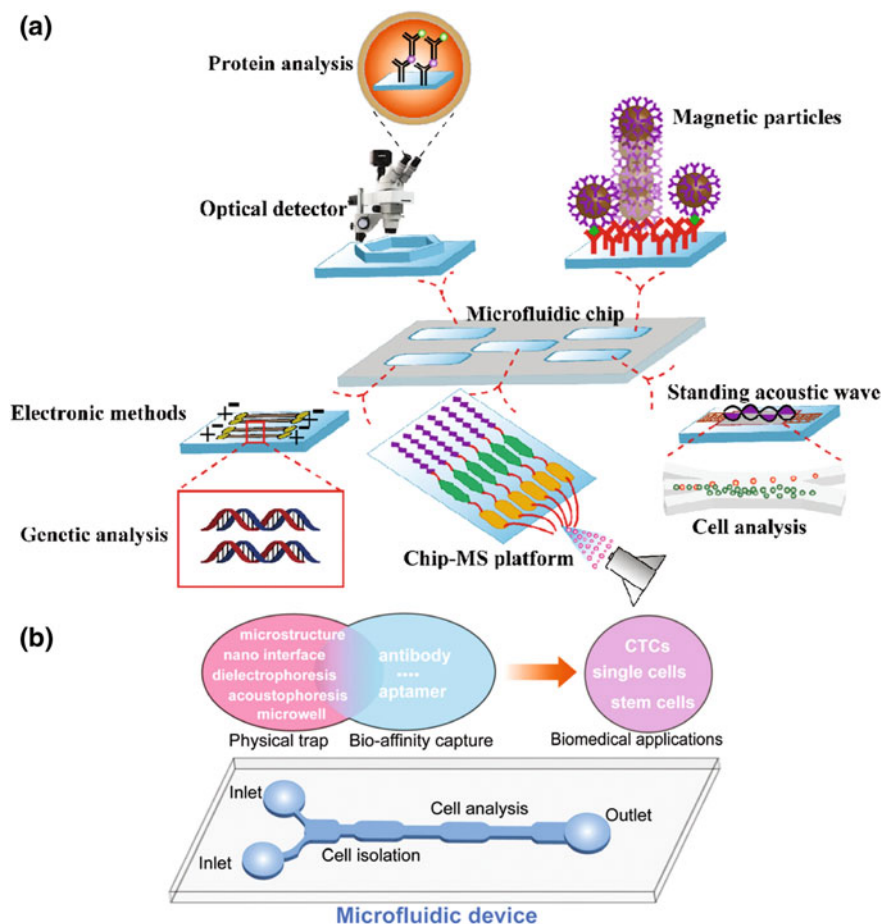


Fig. 1.1 Illustration of lab-on-chip concept. **a** Multifunctional microfluidic platform (Reprinted with permission from Ref. [8]). **b** Microfluidic analysis process for biomedical applications (Reprinted with permission from Ref. [9])

high integration allowing for high-throughput parallel test, miniaturization for good portability, diverse chip designs for various functioning intentions, and precise control of cell culturing environment in cell in vitro models. The processing technique of microfluidic chip originated from the semi-conductor industrial, and was further developed by micro-electromechanical systems fabrication. The wide utilization of microfluidic chip in research fields is benefiting from its analytical ability and manipulation of microscale objects. The fluids dynamics in micro scale is distinct from macro world (Fig. 1.2a) [7]. A variant as Re is used to interpret the characteristics of flow in micro regions. The connection of Re with fluid properties as well as channel dimensions is illustrated in following equation:

$$Re = \frac{\rho v d}{\mu}$$

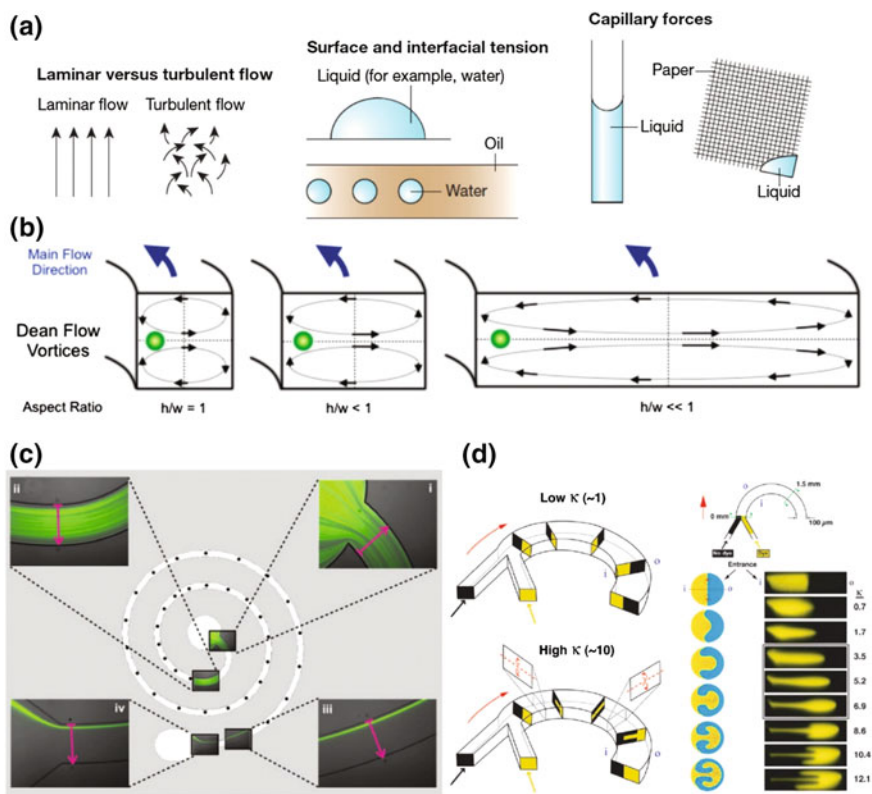


Fig. 1.2 Schematic of flow dynamics in microfluidic region. **a** Laminar and turbulent flow, surface tension and capillary forces (Reprinted with permission from Ref. [7]). **b** Generation of Dean Flow in curved micro channel. **c** Flow focusing in curved channel induced by Dean Flow (Fig. 1.2b) and **c** were reprinted with permission from Ref. [12]). **d** Dean flow enhances flow mixing (Reprinted with permission from Ref. [13])

where ρ represents fluid density (kg/m^3), v stands for average linear velocity (m/s), d for the dimension of channel and μ for fluid viscosity. When Re is larger than 2000, the main characteristic of flow is turbulent with vortexes happening. For cases when Re is smaller than 1, the flow is steady and laminar. In micro channels, Re is basically among the section belonging to laminar flow due to the miniaturized channel dimensions, thus making the flow highly predictable. Lack of convection leads to slow fluid mixing by diffusion. Another difference in microfluidic region is that gravity no longer dominates the object motion as it does in macro world. Surface interfacial tension and capillary forces become two major influencing factors of objects behaviors [10, 11]. Besides, there are Dean flow induced inertia forces (Fig. 1.2b) [12] which can be used for particle focusing (Fig. 1.2c) [12] and enhancing mixing process (Fig. 1.2d) [13].

One of the big future developments for microfluidic chip is to design and prepare function oriented chip prototypes with optimized and facile fabrication steps. And generally, the basic rule for chip design rests in taking the maximum advantage of microscale fluid physics to realize analytical test as well as objects manipulation. Therefore various functional units were invented and integrated into microchip along with its development, such as surface tension valve [14], concentration gradient generator [15, 16] and droplet nozzle [17–19]. To match up the micro analytical system, popular detection methods like fluorescent [20], electrochemical [21, 22] and MS [23, 24] detectors as well as other biosensors [25, 26] were combined to the microchip. In material selection, the choice for chip fabrication evolved from inorganic material of silicon and glass to elastomer, thermoset and thermoplastic, and again further to the paper based and other functional hybrid materials. At present, the microfluidic device has become one of the most popular research tools in analytical chemistry and bio-analytical fields with frequent applications in environmental pollutants analysis [27, 28], food safety [29, 30], disease diagnosis [31, 32] and construction of cell in vitro culture model [33–35].

However, it has to be admitted that the commercialization of microfluidic devices is not successful despite of its broad application in academics and research institutes [36]. Reason for this mediocrity is that comparing to other maturely established detection methods, microfluidic devices are still short of some killer applications, which makes researchers prefer conventional assays than microchips [37]. On the other hand, the microfluidics commercialization is also limited by the difficulties in mass production. The realization of specific functions on chip requires specifically designed chip structure, and it would be overly difficult to format a universal chip model for all kinds of purposes. Commonly researchers would design and prepare homemade chips themselves, especially after the widely utilization of PDMS as a replacement for glass and silicon in chip fabrication and the acceptance of facial and cheap soft lithography as standard preparation methods. In 2013, the market scale of microfluidic devices was about 1.6 billion dollars, with compound annual growth rate of 18–29% to a predicted size of 3.6–5.7 billion dollars in 2018 [38]. The rapid development of this industrial is largely depending on the fast expansion of genome sequencing [39, 40] and in vitro diagnosis applications.

In this chapter, we will have a detailed discussion about the development of microfluidic devices materials and fabrication methods. The design of functional units as well as the field's prospect will also be covered.

1.2 Development of Microfluidic Chip Material

At the primitive stage of microfluidic chip, glass and silicon were the basic materials for chip fabrication. With the invention of new materials and improvement of processing technique, a flood of materials were applied in microchip preparation (Fig. 1.3a) [41]. Currently, the chip materials can be roughly divided into three categories as polymers (Fig. 1.3b) [42], inorganic (Fig. 1.3c) [43] and paper based materials (Fig. 1.3d) [44]. And the material properties are among the basic considering factors for material selection, such as air tightness, biocompatibility, conductivity, transparency and solvent tolerance. For example, glass and silicon are with good stability and highly tolerant to solvents, while the polymer materials have poor resist to solvents, and become partly dissolved or even disintegrate among organic solvents. Besides, the choice of material should also meet the requirement from chip miniaturization and integration. In laboratory environment, the major concern of researchers is how to realize rapid prototype of newly designed chip model and conduct performance testing, but for commercial intention lowering the fabrication cost of product and improving the reliability during usage would be more important. Next, we will have a closer look of the material development history.

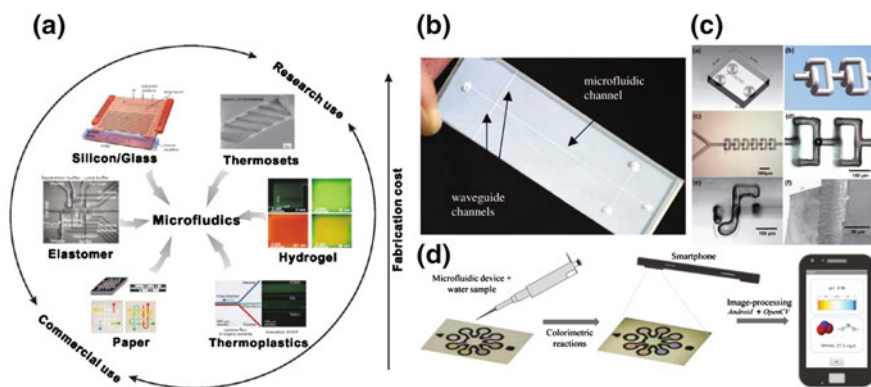


Fig. 1.3 Material choice of microfluidic device preparation. **a** Examples of microfluidic chip material (Reprinted with permission from Ref. [41]). **b** PDMS based micro chip (Reprinted with permission from Ref. [42]). **c** Glass microfluidic channel by laser direct writing method (Reprinted with permission from Ref. [43]). **d** Paper based multiple targets analysis chip (Reprinted with permission from Ref. [44])

1.2.1 Inorganic Material in Chip Preparation

The earliest type of microfluidic device such as miniaturized gas [1] or liquid chromatography [2] and capillary electrophoresis devices was prepared by glass and silicon, under the processing technique inherited from semiconductor and micro-electronics industrials [45]. The advantages of glass and silicon chip are fine chemical stability in high temperature, good strength and thermal conductivity and processability for nano-resolution surface patterns [46, 47].

Silicon is the first material which has been used in microfluidics devices, but was once being replaced by glass and polymers. With excellent thermal conductivity, the use of silicon in some devices is intended to achieve a uniform distribution of temperature [48]. The modification of silicon is easy through the reaction between silylating reagent and silicon hydroxyl exposed on surface [49]. Silicon material has been widely applied in the manufacture of complex 3D structures such as micro reaction chamber [50, 51], cell culture unit [52–54], electrospray nozzle [55] and solvent extraction device [56]. However, the weakness of silicon chip is also significant. Due to lack of elasticity, it is hard to produce flow control elements like pump and valve from silicon. The opacity of material makes optical detection almost incompatible with silicon chip. But still the weakness can be circumvented by comprising hybrid microchip with transparent and elastic material. It is safe to conclude that because of the very appearance of hybrid material chip, the application of silicon in microfluidics is once again expanded.

As contrast, glass is highly transparent with low fluorescence background, which allows for combination with real-time monitoring methods. The thermal conductivity of glass is relatively poor and has been utilized in generation of temperature gradients on device. Fluid flow in glass channel is witnessed with rare non-specific absorption and the modification of glass through silicon hydroxyl reaction is also easy. The air tightness of glass makes it unsuitable for long term cell culture. Glass is well-known for its role as channel material in capillary and chip electrophoresis [57]. Benefiting from high material electroosmotic mobility, fluid in glass capillary is driven by strong electroosmotic flow without the need for external pumping device and being rapidly mixed at good reproducibility. In addition, glass is widely applied in PCR equipment [58] and gas chromatography [59].

Currently, the main processing techniques of glass and silicon material include:

- (a) Standard photolithography [60]. Firstly, photoresist is spin coated to form a thin layer over the surface of glass or silicon, and then exposed to UV radiation against predesigned mask. Next, the patterns transferred from photo mask is developed by developer solution to reveal bare glass or silicon surface for further etching step. The exposed surface is etched by hydrofluoric acid solution for a concave channel. After removal of the photoresist, channels are finally sealed to a plain surface.
- (b) Surface micromachining. As shown in Fig. 1.4a, the fabrication involves steps of patterning sacrificial layer on material surface, casting of amorphous material over the sacrificial layer to form structural layer and etching of sacrificial layer

to achieve the corresponding channels [61]. Comparing to other processing methods, surface micromachining is more capable in fabricating multi-layer devices (Fig. 1.4b) and nano channel due to the minimum thickness of sacrificial layer to a few nano meters [62, 63]. Because multiple steps are required, this machining method would take a long time to finish. Figure 1.4c presents micro featured devices fabricated by surface machining [64].

- (c) Buried-channel techniques. In this method, a tunnel is carved through anisotropic deep reactive ion etching (DRIE) in vertical direction. Then the tunnel walls are blocked by chemical vapor deposition (CVD) only leaving a reaction site for further etching to form a cylinder-shaped channel [65–67]. This method is particularly suitable for preparation of high aspect ratio channel.
- (d) Bulk micromachining. Bulk micromachining is a common micro fabrication method usually employed in preparing micro or nano scale channels [68, 69]. Formation of channel is realized due to the different etching rates for different lattice planes of silicon substrate in electrochemical corrosion. The channel depth is regulated by controlling the etching time, but the accuracy is often unsatisfactory. Another etching method with better accuracy is to dope boron into selected regions, where after doping slower etching rate is witnessed. However the doping process might cause stress in structure and lead to easy break.
- (e) Laser direct writing (LDW). Laser direct writing is a method of fabrication employing laser beam to carve channels directly specially for photosensitive glass material [70–72].
- (f) Electrochemical micromachining. Electrochemical machining was firstly used in aerospace industrial half century ago, and when it was optimized and adapted for fabrication at micro scale region, a feasible method for micro structure building was born. Electrochemical micromachining utilizes cathode and anode to simultaneously and electrically depositing and electrolyzing material at the

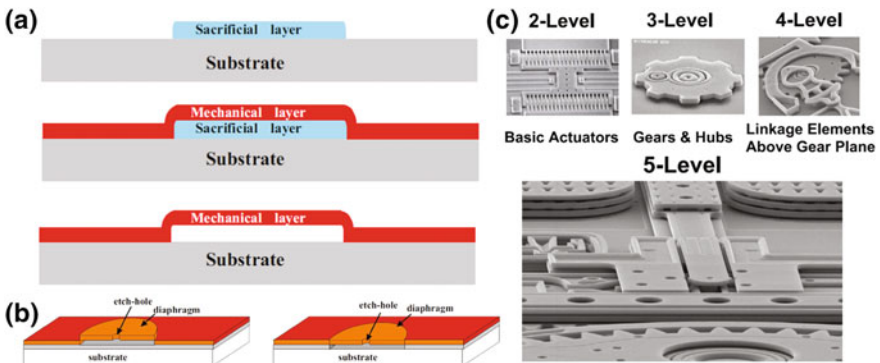


Fig. 1.4 Schematic of surface micromachining. **a** Three steps of surface machining. **b** Illustrations of surface machined devices (**a** and **b** were reprinted with permission from Ref. [61]). **c** SEM photos of surface machined devices (Reprinted with permission from Ref. [64])

same time, and was suitable for processing silicon (Fig. 1.5a, b) [73] and metallic (Fig. 1.5c) [74] material. Combined with computer controlled processor or scanning tunneling microscopy (Fig. 1.5d) [75], high-resolution and three dimensional patterns can be made on material surface with micro scales or even lower dimension (Fig. 1.5e) [76].

Generally the processing steps for glass and silicon based microchip include etching, bonding and connecting to external equipment, and the etching resolution determines the smallest structure that could be achieved on glass and silicon. For glass, wet etching by hydrofluoric acid has been frequently used and resulted in arc shape of the channel side walls [77]. Other etching methods such as plasma etching would be slow and hard to control. For silicon, method of reactive ion beam etching is well established and has been applied in monocrystalline silicon processing to form channels with vertical side walls [78, 79]. Judging from processability of glass and silicon, the disadvantages of their application in chip preparation are high material cost, tedious fabrication steps, employment of dangerous chemical reagents, high standards of bonding condition for chip seal such as high temperature and pressure and ultra-clean environment. Moreover, functional structures like microvalve are hard to be integrated into glass or silicon microchips, which would require additional use of PDMS to assemble hybrid chip. There are other inorganic

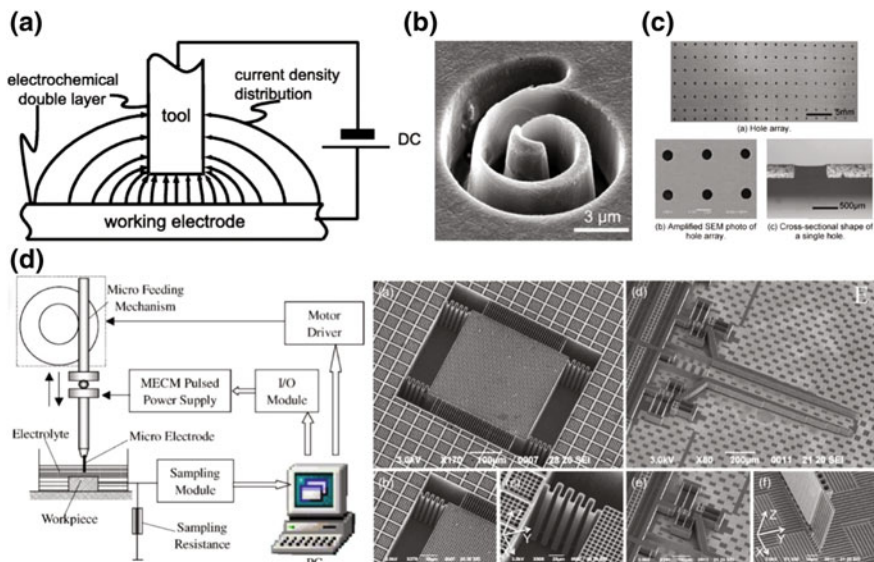


Fig. 1.5 Schematic of Electrochemical micromachining. **a** Principle diagram of electrochemical micromachining **b** Spiral indentation fabricated by electrochemical micromachining (Fig. 1.5a and b were reprinted with permission from Ref. [73]). **c** Through hole array fabricated by electrochemical micromachining (Reprinted with permission from Ref. [74]). **d** Set up of electrochemical micromachining device (Reprinted with permission from Ref. [75]). **e** Electrochemical micromachining of silicon substrate. (Reprinted with permission from Ref. [76])

materials that have been reported in the preparation of microchip, such as low-temperature co-fired ceramic (LTCC) [80, 81], but we will not have a detailed discussion here.

1.2.2 Silicon Elastomer

Polymer materials have been frequently applied to microfluidic devices since 2000s [82, 83]. Comparing to inorganic material, polymers are finely processable and castable, cheap in cost and amendable for mass production. The molecular structure of polymer is variable, and by facile modification its chemical properties can be easily regulated. Polymers applied in chip preparation can be roughly classified into three types, silicon elastomer, thermoset and thermoplastic material.

In the last decade, PDMS has become the primary choice for microfluidics device fabrication [84, 85]. Elastomer allows for rapid prototype of microchip model and assembling with glass substrate for a transparent device. The elasticity makes possible the integration of micro pump and valve into one chip [86–89]. Through placing PDMS layer by layer, complex chip structures can be realized.

Elastomer casting technique was invented by Bell group in 1970s [90], and firstly applied to microfluidic devices in 1980s [91]. After Whitesides reported the PDMS chip fabrication through soft lithography in late 1990s [92], soft lithography as well as PDMS has been broadly accepted by researchers and become a primary chip preparation method. In soft lithography, liquid elastomer is polymerized at a mild temperature condition (40–70 °C), which lowers the infrastructure requirement for laboratory use. The resolution of mold-casting method can reach nano scale, and owing to the small surface tension of PDMS, casted chip device can be easily peeled from photoresist template with good integrity [93, 94]. By simple physical attachment, PDMS channels can be reversibly sealed to another PDMS, glass or other surface. With the assistance of thin layer PDMS as adhering agent or plasma surface treatment, PDMA chip can be irreversibly sealed to another PDMS or glass substrate. Comparing to air tight materials such as glass, silicon, PMMA and PC, PDMS is permeable to gas and permits exchange of oxygen and carbon dioxide in cell culture model [95]. Also the hydrophobic surface of PDMS makes it preferable for cell adhesion [96].

Advantages mentioned above as well as cheap material cost account for the broad application of PDMS in cell biology researches such as cell screening [97], culture [98, 99] and biochemical analysis [100]. And the ability of microfluidics to process samples in nano or pico liter enables the building of single cell analysis platform by PDMS microchips with integration of functional units like micropump and microvalve[101].

However the limits of PDMS are also apparent. Hydrophobic surface of PDMS increases the chances of non-specific absorption of drug components and biomolecules [102]. Although surface treatment by plasma or corona discharge will temporarily reduce this unwanted effect, the treatment often does not last long

[103]. From the view of molecular structure, PDMS is a porous bulk with Si-O chains as skeleton and alkyl groups covering all over it. Therefore, PDMS is extremely vulnerable to organic solvent, and the application of PDMS is only for aqueous system. The air permeability benefits gas exchange but also causes the volatilization of water through micro channel and alternates the solution concentration. Besides, when fabricating channels of high aspect ratio, the flexibility of PDMS material would be more likely to induce channel collapse. The operation condition of PDMS chip has to be mild in prevention of unrecoverable deformation. And due to the existing uncertainties between different baths that may influence product quality, PDMS has been excluded from commercialization.

Substitutions of PDMS are applied to chip manufacture to overcome some disadvantages. For example, perfluoro polymers were introduced for great inertness and anti-fouling properties [104], and hydrogels were used in 3D cell culture matrix and concentration gradient generator[105, 106].

1.2.3 Thermoset and Thermoplastic Materials

Thermoset material was employed in soft lithography as photo resist before it was assumed as device building material. When being heated or radiated, molecules inside thermoset will cross link to form rigid structure, which can't be reshaped once accomplished. Normally, thermoset materials are transparent, stable at high temperature and tolerant of most solvents. Microfluidic chip can be prepared by sole material of thermoset via proper bonding technique [107, 108]. Thermoset materials are proper candidates for constructing complex microstructures through photo initiated polymerization, and the high rigidity permits implement of extreme aspect-ratio channels. However, the high rigidity also makes it impossible to integrate membrane based microvalve on chip. Common thermoset materials include thermoset polyester (TPE) and epoxy plastics.

Different from thermoset polymers, thermoplastics can be shaped and reshaped repeatedly at high temperature. The raw material of thermoplastics is at solid phase when purchased, and when being heated to its glass phase transition temperature, the material is significantly softer and suitable for next fabrication process. Thermoplastics is widely used in modern industrial, typical products of thermoplastics include PMMA, PC, PS, PET, PVC and perfluorinated compounds. Application of Teflon PFA and FEP to the fabrication of microfluidics devices has been reported [109, 110]. Teflon is well resistant to the harmful impact from solvents and chemical reagents, and flexible with good transparency, which can be used for microvalve integration on chip. Generally, thermoplastics have a better tolerance to solvents than PDMS, but are still vulnerable to organic solvents such as ketone and alkanes. The air tightness limits long term cell culture on thermoplastics. Through thermomolding, mass production of thermoplastics based devices can be realized at low cost, which is favored by the commercial companies. However, utilization of thermoplastics in laboratory is limited by the infrastructures of expensive metal or

silicon template. Whitesides and Xia developed a method of transferring patterns from photo resist template to thermoplastics by using PDMS as intermediary material [111]. Restricted by the mild operating condition of PDMS, this method is feasible only with materials of transition temperature lower than 150 °C.

Thermal bonding and adhesive agent assisted sealing are two major packaging methods for thermoplastics, and the operating condition is much milder comparing to glass material. Surface modification of thermoplastics can endure a long time by covalent reaction. For example, the surface hydrophilicity of thermoplastics can last for years after plasma treatment [112].

1.2.4 Hydrogel Material

The skeleton of bulk hydrogel is formed by entangling hydrophilic polymer chains in three dimensions, and there are water molecules occupying nearly 99% of the material space [113]. The porous structures inside hydrogel permit diffusion of small molecules and bio-molecules. Owing to the similarity of hydrogel to extracellular matrix in properties such as permeability and mechanical strength, hydrogels are frequently applied in cell encapsulation and in vitro culture model. However, long-term culture scale is still limited by the effective diffusion distance of nutrients and oxygens, and requires more precisely controlled culturing conditions. The application of hydrogels as supporting material for vascular mimicry model was reported [114], and enhancement of mass transportation was observed [115].

Though hydrogels are generally biocompatible, there are different affinities to cell adhering of different gel materials. For example, animal derived gels such as matrigel and collagen contain factors enhancing cell proliferation, while plant derived gels like alginate and agarose and other synthesized polymers like PEG and polyacrylamide are lack of adhering points for cells. The deficiency of less cell adhering affinity can be overcome by simple grafting modification.

Hydrogel is a low-density soft material, and therefore not ideal for high-resolution manufacturing. The minimum texture in hydrogel is currently at micro scale. Major processing techniques for hydrogel based fabrication include LDW and 3D printing. Due to the mild gelating condition of hydrogel, the only requirement of employed mold template is insolubility in aqueous system. But real problem of gel based devices is the difficulty of sealing. Hydrogels are widely applied in the fields of 3D cell culture and tissue engineering[116, 117].

1.2.5 Paper Based Microfluidic Devices

Microfluidic paper based analytical devices (μ PADs) is an emerging field in rapid development (Fig. 1.6a–f) [118–123]. Whitesides group reported the first paper microchip in 2007 [124], while the prototype of its design can be dated back to

1940s, when Muller and Clegg proposed a method of pH testing by filter paper strips [125]. Muller patterned micro channels on filter paper through paraffin coating and observed plant pigment diffusion and separation over the channel after sample addition of different pigments mix. This very micro channel can be regarded as the embryo of μ PADs. Till now, the applications of paper based devices have expanded to disease diagnosis [126], environmental monitoring [127, 128] and food safety control [129]. Rapid development of paper based devices is based on their unique advantages: cheap and easily obtained materials with flexibility (Fig. 1.6g) [130] and biocompatibility, facile disposal by burning or burying, porous structures and large specific surface area of paper for analytes filtering, transportation and separation, convenient surface modification and patterning by wax coating or covalent reaction, inherent white background for colorimetric detection. Besides, when operating paper based analytical devices, liquids are passively driven by capillary effect without necessity of connecting to external pump, which makes it a proper candidate for portable device fabrication. Functional reagents can be preserved on paper with post bake after addition.

At present, the major directions of equipment development lay on the facile and convenient chip preparation methods and novel chip based test for important biomarkers. Common processing techniques for paper based microchips include lithography [131], inkjet modification [132], plasma treatment [133], direct cutting, patterned wax coating [134], flexographic printing [135], silk-screen printing [136] and laser processing [137]. And the principles are basically the same, which include

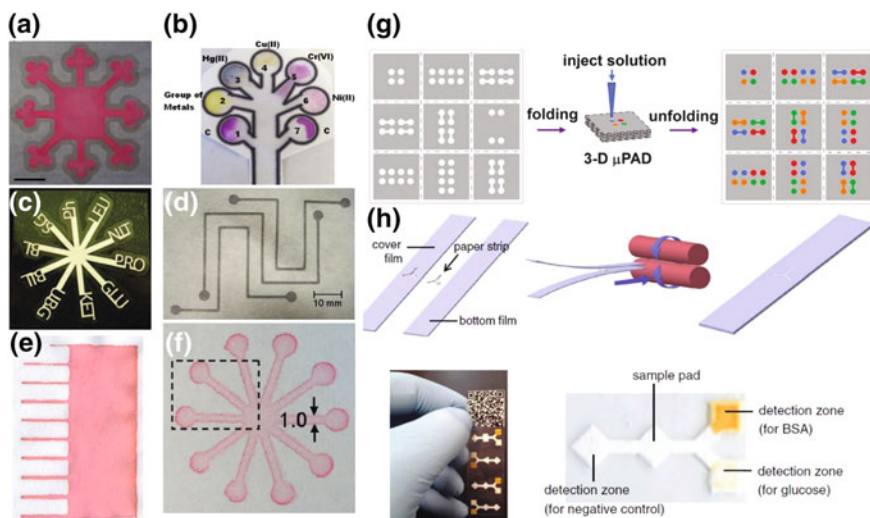


Fig. 1.6 Paper based analytical device. **a–f** Examples of paper based analytical device. (Reprinted with permission from Ref. [118–123]). **g** Origami inspired folding paper device (Reprinted with permission from Ref. [130]). **h** A ready-to use analytical paper strip (Reprinted with permission from Ref. [139])

the formation of hydrophobic/hydrophilic contrast area and restricting liquid flows in predefined microchannels. Normally, the width of micro paper channel is about several hundred or thousand micrometers, and due to the hydrophilicity of paper surface, hydrophobic reagent is employed in paper modification. According to the different cost of surface modification, hydrophobic reagents can be classified into three categories: expensive ones as SU-8 photo resist (modifying 100 cm² requires 0.1 dollars), cheaper ones as wax (modifying 100 cm² requires 0.01 dollars) and the cheapest ones like alkyl ketene dimer (modifying 100 cm² requires 0.00001 dollars). And modification principles can also be differentiated as physical block of surface porous structures, deposition of hydrophobic layer and chemical regulation of surface property. Modification by chemical reactions can be hardly removed by solvent dissolving, while physical deposition on surface is vulnerable to organic solvents, which is utilized in channel formation on paper based devices.

The processing techniques for surface modification include hydrophobic coating in predesigned patterns such as wax printing and ink jet printing, and overall coating followed by selective removal of coating material through lithography, microfluidics methods and region confined plasma treatment. Currently, the most frequently used methods are ink jet printing of AKD reagents and wax coating. Common advantages shared by these two techniques are low in cost, easy to control and short fabrication periods.

Detection methods accommodated with paper based microfluidic chips include colorimetric, electrochemical, chemiluminescence and electrochemiluminescence detections. Among all these methods, colorimeter has been often applied to qualitative and semi-quantitative testing due to its direct observation by raw eyes of the reaction products under enzymes catalyzed systems [138]. And because of no requirement of external equipment, colorimetric methods have also become well commercialized to ready-to-use products (Fig. 1.6h) [139]. Electrochemical methods have high sensitivity which is capable for quantitative detection of substances as low amount as nM level. Besides, the application of electrochemical detection can also effectively avoid the interference from other colored matters in detection matrix [140]. Studying from the published works, the major detection methods employed in paper based devices are colorimetric and electrochemical, while in few works chemiluminescence and electrochemiluminescence were applied.

Future development directions for paper based devices lay on the studies of inventing cheap, easily-handled and portable devices to help improve diseases diagnosis, especially in the developed regions which suffer from the lack of infrastructures and trained medical workers. Also development of quantitative detecting papers of multiple targets on the basis of current qualitative and semi-quantitative test papers is in avid demand. At present, the functional test papers can be roughly divided into two types: on demand devices and ready to use devices. Different reacting reagents can be added to the former ones according to different target substances, while for the latter ones, specific reagents for single target test have been placed into the paper in advance and simple sample addition can lead to detection results.

To reinforce the detection capability of paper based devices, integration of different functional units is needed, such as sample separation channel and reaction chambers. The development of 3D paper chip has allowed the transportation of solution not only inside 2D plane, but also in vertical directions [141]. The simplest method for 3D paper chip fabrication is physical folding and overlaying of 2D paper chip. Comparing to 2D chips, the appearance of 3D chips has largely increase the device detection throughput, where each layer can be designed to implement analysis for one specific substance. Of course, one popular application for paper chip is as supportive material for cell biology related researches. For example, paper chip has been applied as 3D cell culture matrix for in vitro model construction [142].

As it is said, every coin has two sides. The limited factors for paper based devices including:

- (a) The diffusion and carryover of sample inside paper would significantly decrease the valid concentration of target for detection. Also open channels on paper would enhance water volatilization and harm sample transportation. Normally, amount of sample reaching detection area is no more than 50% of the sample addition.
- (b) For fluids with low surface tensions (bio-sample containing surfactant, for example), hydrophobic modification on surface is not enough to confine sample solution inside predesigned channels. In this situation, sample channeling is not effective by controlling surface hydrophilicity/hydrophobicity.
- (c) Frequently used in conventional methods, microfluidic hydrodynamics such as laminar and micro droplet technology can't be accommodated to paper devices. Micro structures like micro valves are unable to be integrated into paper chip.
- (d) Detection limit of colorimetric method is not low enough for trace amount pollutants in drinking water, which is usually in ppb or ppt level.

1.2.6 Hybrid Material Chip

When single material is unable to fulfill all the technique requirements to realize device function, coupling different types of materials in one microchip should be considered. We term the chip which extensively employs two or more types of materials the hybrid chip. Design of hybrid chip should following the principle that combined materials can't antagonize each other, but serve as complements to improve over all properties. One typical example of the hybrid chip includes glass as substrate and PDMS as channel and microvalve formatting material in one microchip [143–145]. There are also other examples such as glass channel and electrode substrate [146], and microchannel embedded with hydro gel to eliminate bulk flow while preserving diffusion.

1.3 Chip Fabrication

We have had a brief introduction about the chip materials and different fabrication methods that vary according to material as mentioned above. Next, we shall supplement some frequently used chip fabricating methods to make it more complete.

1.3.1 *Soft Lithography*

After the elastomer became the major material for microfluidic devices preparation, its fabrication methods have also been developed since. Currently the basic method for PDMS chip preparation is soft lithography. Soft lithography was invented in the late 1990s by Whiteside's group [92], and its principle originated from standard lithography which is employed for glass and silicon chip preparation. In standard lithography, glass or silicon surface is covered with a layer of photo resist, which is selectively exposed to UV radiation and developed. The bare surface without photo resist covering is vulnerable to chemical etching, and thus ready for channel formation. For soft lithography, elastomer is mold-casted to a photo resist template standing on silicon base. After elastomer solidification under hours' heating, the whole PDMS bulk is peeled from the template and sealed to a glass substrate. Main processing steps of soft lithography include: UV mask design, photo resist spin-coating on silicon wafer, exposure to UV and development, mold-casting and sealing. With the advantages of low infrastructure cost and simple operation, soft lithography has been widely applied in laboratories of universities and institutes to achieve fast prototype of chip model. However, the disadvantages are multiple processing steps. From preliminary design to real products, it often requires several hours to days to cover a single fabrication period. If the mold chip couldn't pass performance testing, preparation would have to restart over from design, which makes a great liability on time and labor. Limited to the lithography principle, some three-dimensional structures are unable to be formed. In the aspect of resolution, no matter the specific lithography processing steps, the final resolution is always limited by diffraction effect. Slight differences may be observed by using different masking technologies. For example, resolution of contact photolithography is about 0.5 μm but 0.3 μm for projection photolithography [4]. Generally, microchip prepared by lithography can meet the requirement of micro volume bio-sample analysis, but when more precisely controlled structure feature is needed, electron beam etching or nano imprint lithography may be necessary. Both of these methods can lower down resolution to below 100 nm, but with heavy cost of equipment and time.

1.3.2 Fabrication of Extended-Nano Channels

The scale of extended-nano channel (10–100 nm) lies between the conventional nanotechnology (1–10 nm) and microtechnology (>1 μm). In the past, researches concerning fluid properties at extended-nano scale were limited by the lack of appropriate tools. Extended-nano channel is too large for bottom-up synthesis or assembling from atoms or molecules of nanotechnology, and too precise for micromachining of microtechnology. Therefore, specialized methods are required to study channels at extended-nano scale. Focused ion beam milling and electron beam lithography have become alternatives to traditional photo lithography and applied to nano channel formation (Fig. 1.7a) [147]. These methods are able to realize precise nanomachining in selected regions, but unsuitable for large scale and high throughput fabrication. Focused ion beam milling mainly uses gallium ion beam to directly carve 2D nano channels on silicon or fused silica. Normally, the formed extended-nano channel is embed and connected with surrounding microchannels to constitute complete flow system. The microchannels are intended for solution supply.

In electron beam lithography, electron resist and conductive polymer are spin coated to quartz wafer respectively. Nano patterns are created by electron beam drawing without the requirement of photo mask. Then with the coupled plasma and chemical vapors treatment, extended-nano channels are achieved on substrate. And chip sealing is accomplished by thermal bonding with or without external pressure. In addition, laser direct writing can also be applied for fabrication of extended-nano channels (Fig. 1.7b) [148].

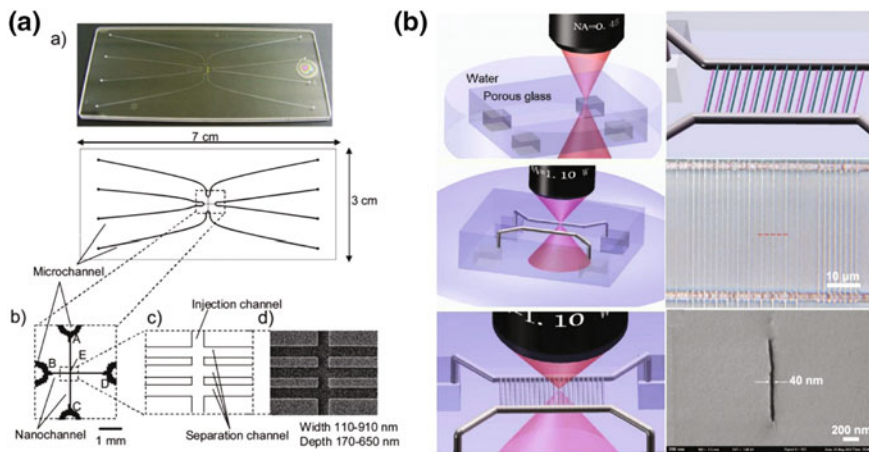


Fig. 1.7 Extended-nano channel fluidics. **a** Extended-nano channel produced by focused ion beam milling and connected to micro channels (Reprinted with permission from Ref. [147]). **b** Extended-nano channel bunch fabricated by direct laser writing (Reprinted with permission from Ref. [148])

1.3.3 3D Printing Technology

3D printing technology firstly appeared in the 1980s [149], but subject to expensive equipment, limited printing material and few choices of printing nozzle, the application of 3D printing technology in microfluidics was only restricted to chip prototype and exploring fabrication methods. Thirsty years since then, as the development of technology and decrease of equipment and material cost, 3D printing has not only been widely employed to the researches of various disciplines in laboratory, but also come to the daily life of ordinary customers. Customer grade 3D printing device has now been successfully commercialized with price at 1000 dollars [150].

3D printing is a highly precise manufacturing method, enabling facile and easy preparation of complex 3D structures. Comparing to conventional microfluidic device fabrication methods like lithography and micromachining, 3D printing is simpler in operation, enables full automation and on-step fabrication of microchip. 3D printing spares the design of UV mask, and testing performances can be directly used as reference for design optimization, thus increasing the speed of experience based modifying. At present, the choice of materials for 3D printing is broadly extended, and materials from polymers to various bio substances such as cells [151] can be printed.

3D printing is also called additive manufacturing (ASTM F2792), that is, device manufacture by continuous addition of new material to substrate. Commonly, steps of 3D printing include:

(a) Building 3D model of target object on CAD or other engineering drawing software. (b) Transferring the model data to 3D printing devices where 3D model is sliced to be successive 2D layers. The final amount of 2D slices is determined by the resolution of 3D printing devices, and normally printing resolution can be smaller than 10 μm .

(c) Layer by layer 3D printing of material to rebuild the 3D features of model objects. (d) In construction of some structures, post-processing is needed to remove the supporting frames or other exogenous materials. 3D printing devices are equipped with precise positioning and delivering modules, which can build structures of any shape in 3D space theoretically, like curved surface, slope and irregular shape that can't be prepared by other methods.

According to different printing material and polymerization methods, principles of 3D printing can be further classified as stereolithography (SLA) (Fig. 1.8a, b), fused deposition method (FDM) (Fig. 1.8c), electron beam melting (EBM) and bio-printers, the first two of which have been well exemplified [152].

1.3.3.1 Stereolithography

Stereolithography was the principle of both the first proposed 3D printing device and the first batch of commercialized products of 3D printing, and still the most

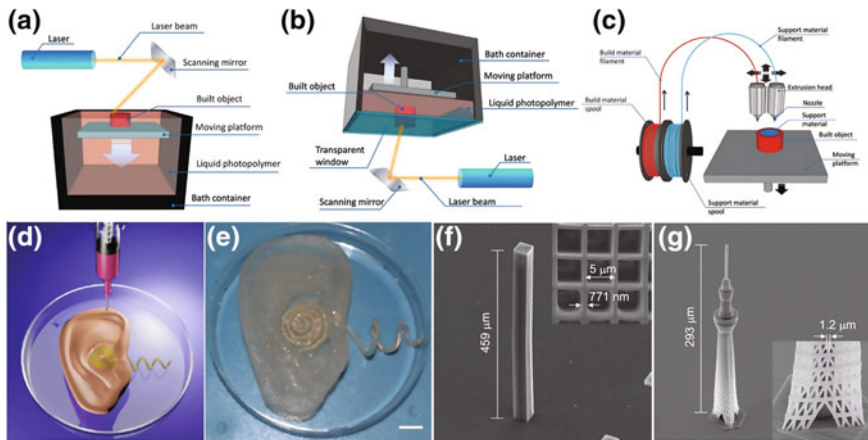


Fig. 1.8 Schematic illustration of **a** Bath configuration of stereolithography, **b** Layer configuration of stereolithography and **c** Fused deposition modeling. (Figure 1.8a–c were reprinted with permission from Ref. [152]). **d** Computer model design of human ear. **e** 3D printed ear. (Figure 1.8d and e were reprinted with permission from Ref. [157]). **f** and **g** 3D printed structures (Fig. 1.8f and g were printed with permission from Ref. [158])

widely used and successful models. In 1988, Chuck Hull invented the first 3D printing device by using UV laser to initiate liquid resin polymerization and localized solidification. The vertical dimension of object was expanded by the lifting of carrier platform. During printing, fresh resin was painted to the section and then shaped by laser beam. And above procedures were repeated until the recapitulation of 3D objects. The vertical resolution of stereolithography can reach sub micrometers or tens of micrometers, while the planar resolution is determined by the size of laser beam spot. Currently, single layer of resin being painted is 25 μm in thickness with minimum laser beam shifting distance of 10 μm . In addition to laser beam, digital light processing projector and LED can serve as light sources.

The advantages of stereolithography are precise patterns on 2D plane, modest consumption of liquid material, low cost in printing and ability to produce customer grade 3D printing devices. However, limited by printing procedures, only one epoxy or acrylate resin can be applied during one printing operation. And requirement of post-processing may be another disadvantage of stereolithography. Besides, the printing process may be influenced by uncontrollable non-linear factors such as resin polymerization around the vicinity of laser spot and temperature shift induced polymerization. Based on the mechanism of stereolithography, researchers developed multijet or polyjet technique to print multiple photo sensitive inks for UV induced polymerization [153, 154]. Multijet technique has equivalent or even better resolution comparing to conventional stereolithography, and employment of multiple nozzles enables switch of materials during printing. The major defect of this method is expensive hardware cost, which corresponds to 10 or 100 times of the cost of customer grade printing devices. Besides, other derivations based on stereolithography include digital micromirror device-based projection printing

(DMD-PP) and two photon polymerization (2PP). Both of these two methods have better printing resolution than stereolithography, but the time required by processing is prolonged.

1.3.3.2 Fused Deposition Method

In fused deposition method, melted thermoplastic material of several millimeters diameter is squeezed out from a tiny nozzle of hundreds micrometers wide and added to the printing structure [155]. Common printing material include polylactic acid, polycarbonate and acrylonitrile butadiene styrene (ABS), and through mixing stainless steel particles, wood chips, ceramic powder, carbon nanotubes and graphene into thermoplastics, hybrid materials can be obtained. After squeezing, printing material is piled up shoulder by shoulder in the shape of cylinders, therefore resulting in larger roughness than other 3D printing methods. Certainly, fused deposition method has its own unique advantages, including compatibility with various cheap and easily obtained materials, low cost in equipment, alternative printing of several materials by integration of multiple nozzles and cost performance allowing for development of customer grade devices. Similar with fused deposition method, direct ink writing (DIW) uses viscoelastic ink with regulated rheological properties and pneumatic pump controlled injection to directly paint material to substrate [156]. For DIW, the minimum diameter of nozzle lumen can be 1 μm , and normally used materials are ceramics, polymers and metal nano particles.

When assessing the performance of a 3D printing device, major concerned parameters are resolution, printing speed, material compatibility and manufacture size. And there is huge space for further development of current printing devices in these aspects. As for hardware, cost of printing devices is mainly ranging from thousands or tens of thousands dollars and it is necessary to further cut down the cost of equipment. For printing material, the choice is largely limited by the processing procedure, and functional properties of material like biocompatibility and transparency become subordinate factors. In the future, 3D printing technique should provide more options when it comes to material choices and strategies for multiple materials alternative printing (Fig. 1.8d–g) [157, 158]. Finally, the resolution of 3D printing needs to be improved comparing with soft lithography [159]. And it is a large challenge to avoid significant prolonged time period when increasing printing resolution. One feasible manner is to combine high resolution printing and low resolution printing into one device to balance the time cost and requirement of printing resolution [160]. Of course, the incoming problem is largely increased cost of equipment to implement two printing system in one machine.

1.4 Integration of Microfluidics Functional Units

The miniaturized features of microfluidic chip make possible not only the manipulation of micro objects and analysis of micro volume sample, but also the integration of different functional units into one chip. Functional units on chip can realize sample pretreatment, flow control, cell culture and sensing detection, which greatly extend the detection capability of device. Next, we shall have some introduction about the common functional units in microfluidic chips.

1.4.1 *Flow Manipulation: Micropump, Microvalve and Mixer*

In microfluidic devices, flow control can be divided into two types according to the underlain principles. The first type of control relies on the physical effects that are significant in micro scale such as surface tension and capillary effect, and it is called passive manipulation. The second type depends on external forces like pump to control flow, and is called active manipulation. Common driving forces for flow pump include electricity [161], magnetofluid power [162] and electroosmotic flow [163]. Besides, flow driven by centrifuge force from device rotation is also subject to active manipulation. Pump has been regarded as the pivot for flow system, and when building microfluidic chip based analytical platform in laboratory, external pump is often essential. The advantages of connecting a pump are obvious: precise and steady control of flow and realization of complex programmed pump behaviors. But in development of mobile device, external pump would harm the device portability, and extending conductor pipe would cause dead volume and result in sample waste. There are publications reporting integration of micropump on chip, and according to different driving manners, passive or active pump is called (Fig. 1.9) [164]. Principle of passive micropump is the capillary effect, while the active pump has variety of driving forces, such as electroosmotic, electrochemical, pneumatic peristaltic, acoustic, electrostatic and magnetic power. Electroosmotic driving provides steady flow rate, and through switching the polarity of electrode, flow direction can be easily reversed. Owing to mechanical-motion-free feature of electroosmotic unit, this type of pump can be simply integrated into micro chip [165]. Electrochemical pump relies on the generating bubbles from water electrolysis to drive flow forwards [166], and pneumatic pump depends on the pressure induced deformation of membrane to control the flow in lower channels [167].

Pump provides driving forces for fluid flowing, while valve is in charge of the on-off of flow circuit. Microvalve on chip can also be divided into two types as passive and active valve. Passive valves refer to those independent of mechanical opening and closing, the typical examples of which include cantilever valve, diaphragm valve and diffuser valve. Active valves are oppositely dependent of mechanical forces for the valve switching, and may be based on the principles of

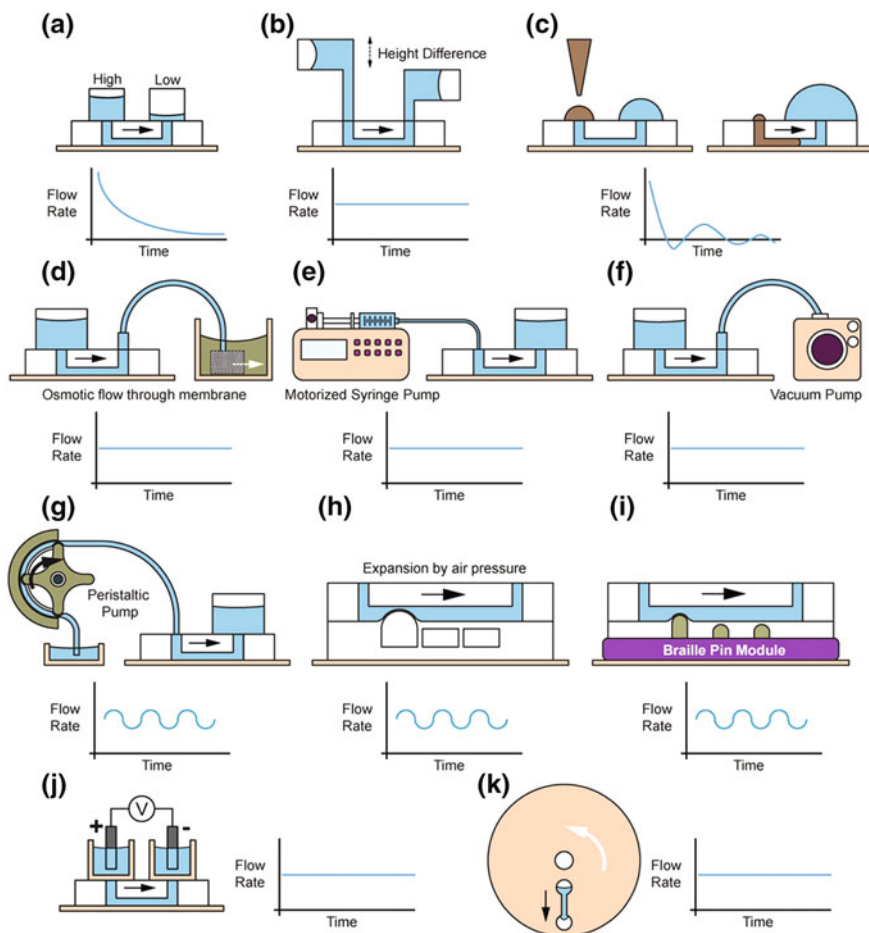


Fig. 1.9 Schematics of different type of pumps for microfluidic devices. **a, b** Gravity driven pump. **c** Surface tension droplet pump. **d** Osmosis based pump. **e** Syringe pump. **f** Vacuum pump. **g** Peristaltic pump. **h** Pneumatic peristaltic pump. **i** Braille pin peristaltic pump. **j** Electrokinetic pump. **k** Centrifugal pump. (Reprinted with permission from Ref. [164])

piezoelectric ceramics, thermal pneumatic power, electrostatic forces and electromagnetic interaction. Currently, pneumatic membrane microvalve prepared by PDMS is the most widely used integrated valve in microchip [168].

In the field of microfluidics, the tiny scale of channel as well as the laminar makes solution mixing a very slow process basically through free diffusion. And this is an unfavorable fact for mixing, but has been ingeniously applied for the generation of concentration gradients. Methods to enhance flow mixing include producing convection and shortening diffusion distance. For example, protruding geometries were designed inside micro channel to effectively induce turbulent flow and enhance solution mixing, which is easy to realize on microchip [169]. Besides, external forces have also been employed to increase the turbulent component of fluid flow.

1.4.2 Concentration Gradient Generator

Biomolecules regulate the behaviors of cells such as proliferation and differentiation among inside-body environment. There are researches indicating the close relation between the concentration gradient of cellular factors and cancer invasion [170], and this chemotaxis of cancer cells has been employed to develop anti-cancer medicine. Conventional drug concentration screening experiments are conducted on multi-well plate, and as the growing maturity and broader application of microfluidic technique on biological researches, methods of concentration gradients based on microfluidics have been rapidly developed. Advantages of concentration gradients on chip include wider gradient ranges, more precise partition of concentration on space distribution, maintained gradient persistency and compatibility with real time monitoring method of cell behaviors. Generally, the strength of chip based concentration gradients reflects its powerful control of the cell culturing environment. And until now, concentration gradients have played important parts in the researches of wound healing [171], inflammation response [172] and cancer metastasis [173] as well as chemical synthesis of drug. According to its time dependent stability, concentration gradient can be classified as dynamic and static. Major methods for the concentration gradient realization on chip include:

- (a) Semi-permeable membrane diffusion based concentration gradient. As mentioned above, fluid mixing through diffusion inside a tiny channel is very slow, which provides chance for concentration gradient creation (Fig. 1.10a) [174]. To preserve diffusion based concentration gradient, the priority is to eliminate the convection flow through methods such as setting up semi-permeable membrane to separate two flows [175]. Another method is to deploy narrow connecting channels between different chambers (Fig. 1.10b) [176]. However, normally the gradient can't last long.
- (b) Convection based concentration gradient generator. Convection is actually also applied to create concentration gradient. Typically, convection requires rather higher flow rate and precise flow control, which may cause harm to cell biology due to the large shear force. On the other hand, running flow is critical for the medicine transportation and removal of metabolic waste. Therefore the flow rate can't be too small. According to the equation of shear force,

$$\tau(\text{cell}) = 6Q\mu/h^2w$$

shear force is related to flow volume rate, fluid viscosity and channel dimension (height and width). Increasing the channel height and lowering flow linear velocity can help reduce shear force subject to cell and alleviate cell damage. Another feature of convection based concentration gradient is that the

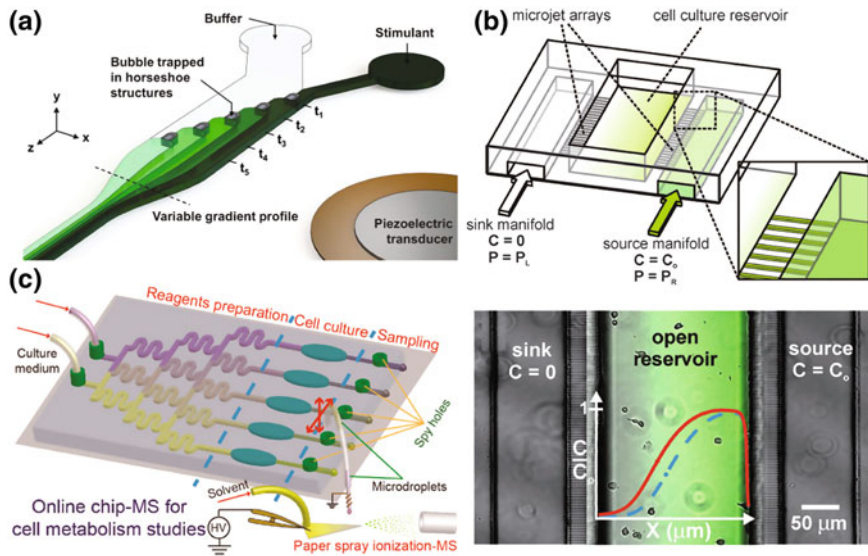


Fig. 1.10 Concentration gradient generator. **a** Concentration gradient produced in a Y channel with multiple barrier units (Reprinted with permission from Ref. [174]). **b** Drug concentration gradient controlled by narrow connecting channels (Reprinted with permission from Ref. [176]). **c** Tree-shape concentration gradient generator. (Reprinted with permission from Ref. [15])

formation of gradient is always perpendicular to flow direction, for example the well-known “Christmas Tree” model (Fig. 1.10C) [15]. Christmas tree shaped gradient generator was proposed by Jeon in 2000 [177], and by regulating flow rates and concentrations of different flows, concentration gradients of special and temporal variety can be created. Convection based gradients can be preserved for a long time, and similar structures include Y-shape channel.

- (c) Hydrodynamic focusing device. Through hydrodynamics related method, externally added drugs or cell factors are confined in a predesigned area [178].
- (d) Concentration gradients on micro droplets. Droplet technology is a unique product born along with the development of microfluidics and has been applied in micro-volume reaction, single cell analysis and material synthesis. Regulation of concentration in the dispersion phase can achieve a gradient distribution over different droplets [179]. The combination of droplet technology with concentration gradient method avoids the external interference and provides a stable culturing environment for cells. Besides, the application of droplet technology greatly improves the analysis throughput, which can be further applied in drug screening.

1.4.3 Cell Culture Chamber

Integration of all kinds of functional units on chip makes microfluidic chip a platform for various research purposes rather than a specific analytical device. In biology related researches, the functions of microchip is extended from pure analysis of cell lysis and metabolites to construction of cell in vitro culture model, studying intercellular interaction and single cell analysis. And one critical step involved in the realization of above functions is the cell online culturing. Compared to conventional cell culture methods, advantages of using microfluidics rest with the precise control of microenvironment and targeting smaller cell population even single cell as research subject. Cell culture experiments in petri dish normally ignore the distinctions between different cell individuals, but rather take averaged responses from mass amount of cells as final results. In some situations, research concerning single cell behavior bears larger significance, for example the study of stem cell proliferation and differentiation under certain circumstances [180]. And at this point microfluidic chip has become one ideal candidate from not many available choices for single cell research. Series of derivative technologies concerning cell manipulation have been developed such as 3D hydrogel encapsulated culture and microwell capture array, which we will discuss in the later chapters.

1.4.4 Integrated Biosensors

When establishing microfluidic chip based analytical platform in laboratory, optional external detectors include mass spectrometer, fluorescence monitor and electrochemical detector. However, for analysis of complex biosamples, common detecting methods usually fail to achieve high specificity and sensitivity. And to solve this problem, specific biosensors are required and integrated into microchip to improve analysis efficiency (Fig. 1.11a, b) [26, 181]. On chip biosensors can be categorized as enzyme catalyzed reacting biosensor, immune biosensor, optical detector and DNA detecting array. Integrated biosensors can effectively reduce the consumption of sample and reagents, shorten operation time, lower detection cost and help development of portal devices. We shall have a brief introduction of on chip biosensors in the following content.

1.4.4.1 Enzyme Catalyzed Biosensor

For enzyme biosensor, enzymes with bioactivity are fixed inside channel or to the electrode surface (Fig. 1.11c) [182]. When passing through detection area, the analytes are catalyzed by enzymes, transferred to color changes or electrochemical signals (Fig. 1.11d) [183] and detected. At present, enzyme biosensors are widely applied in the detection of blood glucose, urea and cholesterol, among which glucose assay is the most successful commercialized product. The first glucose

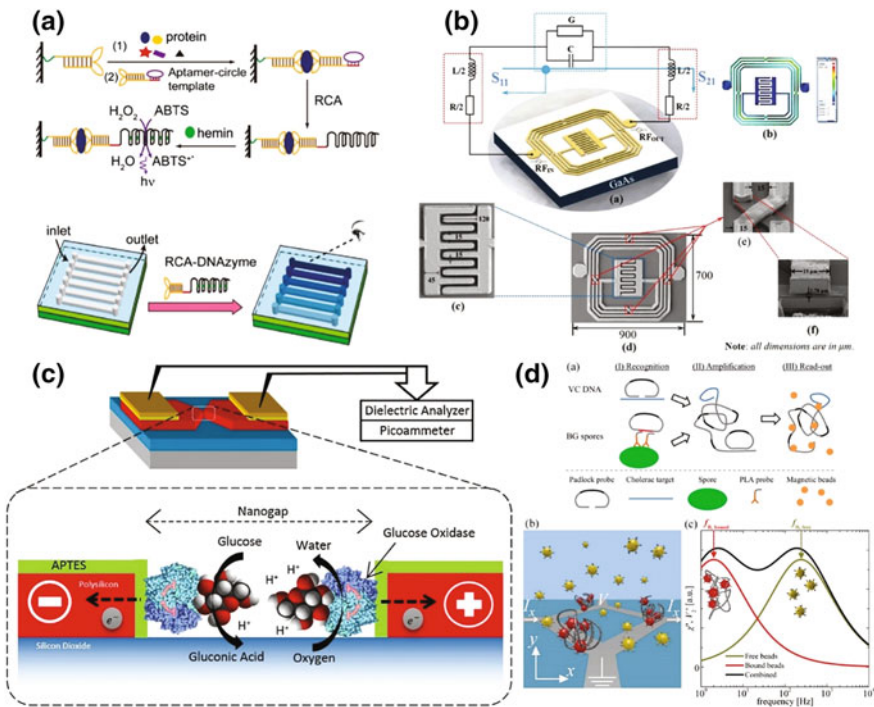


Fig. 1.11 On chip integrated biosensor. **a** Biosensor based on aptamer recognition. (Reprinted with permission from Ref. [26]). **b** Circuit design of an electrode based sensing chip (Reprinted with permission from Ref. [181]). **c** A dielectric analyzer for glucose detection (Reprinted with permission from Ref. [182]). **d** DNA recognition and analysis on bio-sensing chip (Reprinted with permission from Ref. [183])

biosensor was invented in 1962 [184], and decades since, there had been many optimized versions while with little alternation of the basic principle of electrochemical detection by enzyme modified electrodes. Urea level in blood is also an important indicator of many diseases like renal failure, nephritis, urinary obstruction and gastrointestinal bleeding. For urea detection, urease and glutamic acid dehydrogenase are employed.

1.4.4.2 Immune Sensor

Immune sensor is a detection method based on antigen-antibody recognition and turns the existence of analytes into fluctuation of resistance, electric current, electric capacity and refraction index. Specificity of immune sensor is high with good reproducibility. But the limits are high cost of monoclonal antibodies and tedious detection cycles. Immune sensors are mainly used in on chip detection of pathogen, bacteria, and viruses.

1.4.5 Microfluidic Centrifuge Device

A special type of device with the shape of laser Discs is invented with the development of microfluidics and called microfluidic centrifuge device (Fig. 1.12a–d) [185–188]. During operation, the high speed rotation of device generates centrifuge force to drive sample flow into treatment chamber, without the need of connecting to external pump. History of centrifuge device can be traced back to 1960s, when ORNL micro analytical device was employed in clinical to transport sample solution by centrifuge driving. But the idea of functional integration and miniaturization hadn't come to stage back then. After decades' development, microfluidic centrifuge device has become a very important tool for sample treatment and analysis, especially in the fields of clinical detection, immune diagnosis, protein analysis, cellular sample handling and biomarker detection.

As mentioned above, microfluidic device spares the trouble of connecting to an external pump and allows for set up of enclosed system. Due to the removal of external conductor system and accompanying dead volume, waste of sample is avoided. Centrifuge device is capable of processing sample in the volume of nano liter to milliliter, and the driving of flow in centrifuge device is continuous due to the rotation movement of device. Other unique functionalities reflected by centrifuge device include density based components separation, automatic processing

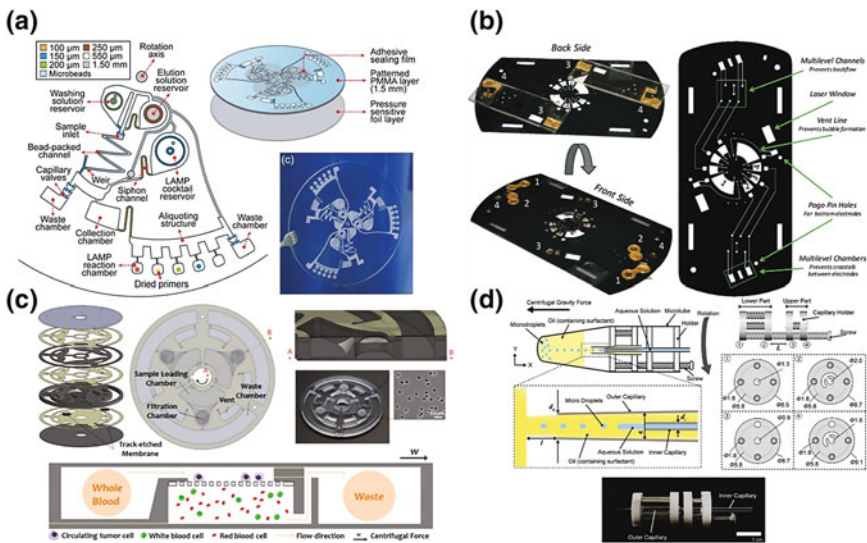


Fig. 1.12 Examples of centrifuge device. **a** Schematic of colorimetric foodborne pathogen detection on centrifugal device (Reprinted with permission from Ref. [185]). **b** Centrifugal device for electrophoretic separation of DNA (Reprinted with permission from Ref. [186]). **c** Centrifugal device for tumor cells separation from blood sample (Reprinted with permission from Ref. [187]). **d** Centrifugal device for droplet production (Reprinted with permission from Ref. [188])

procedures such as sample inflow and eviction under different rotation speeds and miniaturization and integration. Generally, the working procedures of microfluidic centrifuge device are sample addition, reagents storage and release, liquid transportation, valve switch, mixing and detection. Direction of liquid transportation is normally from center of the device to outer margin due to the centrifuge force driving. Therefore, the sample inlet is also placed in the center of the device. Detectors compatible with centrifuge device are mainly optical and electrochemical. Although numerous companies have participated in the development of microfluidic centrifuge devices, few commercialized products have come into the market. The reasons for this weak commercial transformation are in two aspects. In one hand, employment of centrifuge movement saves part of the trouble in developing portal device by removing external pump, but also results in extra requirement for external power supply. Therefore, centrifuge device doesn't actually improve portability. On the other hand, flow control by centrifuge force is apparently not as precise or stable as flow pump does, and special liquid transporting manner causes the chip design some problems.

1.5 Development and Outlooks

Going through the brief history of microfluidics development, we have witnessed a path of favored diversity. In the choice of chip materials, there were different times when inorganic and polymers taking the dominant positions. In specific formats, there are ready-to-use test papers and analytical platforms with multiple functionalities. Actually when speaking to the future development of microfluidics, it is hard to cover all kinds of devices by a single standard. Each microfluidic device has its own development according to the different functionalities. Next, we will focus on several emerging fields of microfluidics which might have important applications in the future.

1.5.1 *Point of Care (POC)*

Analytical methods of series of biomarkers have been well established in laboratory, which require extensive work as well as professionally trained personnel. The common procedures include sampling, detection and results analysis. To improve the convenience of biochemical test and shorten detection time, point of care detection methods based on microfluidics technology have been developed. The major detection targets of POC methods are small molecules (blood glucose and urea), cells (CD4 + T-cell counter for AIDS diagnosis) and nucleic acids. The purpose of POC diagnosis is to make preliminary screening test closer to patients and may be used in community medical center, clinics, emergency center or at home.

Although microfluidic methods receive mass attention in academic circles and funding supports from government and private capitals, current examples of commercialization of POC device are still few. The major defects of POC device are the compromised sensitivity and quantification ability in analysis of complex biosamples comparing to laboratory large equipment. The development of POC device largely depends on the discovery of new disease biomarkers, especially those existing in the peripheral body fluids. For certain diseases such as cancer, diabetes, AIDS, tuberculosis and Alzheimer's disease, positive results have been obtained in biomarkers discovery, but there is a lack of unified standard and theoretical basis for biomarker based disease diagnosis. At present, commercialized POC devices can be classified into two types, and one is the lateral flow test that uses test paper or semi-permeable membrane strip for protein detection. The scale of global market for in vitro diagnosis has exceeded 40 billion dollars and 1 billion among is occupied by test paper business. Normal working procedures for test paper are sample addition, solution flowing through areas with labeling reagents and capture array, and finally formation of a colored line by labeled and captured biomarkers which can be told by raw eye observation. Typical examples are pregnancy test and flu test. In areas suffering from lack of infrastructure, test paper is also used for HIV diagnosis. Lateral flow based test is capable for target analysis at μM or mM level. Lower amount of analytes requires specific amplification strategies for detection, which may increase the complexity of device structure. In the last decade, plenty of funds and manpower have been invested to improve test paper performance, but little payback is obtained.

Another type of POC device is the electronic detecting instruments represented by glucose meter. Commercialization of glucose meter is an unrivaled success, which can be hardly duplicated by other products. There are unique favorable factors for the market expansion of glucose meter. Firstly the content of blood glucose in human body reaches mM level and exceeds the lower detection limit of electrochemical methods. Secondly, frequency of glucose meter usage can be several times a day for patients of diabetes, which provides large demands for the consumptive test papers.

There are problems needing to be solved in the future development of POC device.

First question is how to integrate multi-step sample treatment and analysis into one device and realize full automation of processing from samples to diagnosis results. Procedures of analysis include sample collection, pretreatment, analyte reaction, signal transformation and report. Due to the vast disparities of technologies involved in different steps, it is hard to meet all requirements by one scheme.

Other questions are how to simplify operation of device and make untrained personnel capable of operating, and how to improve test reliability.

1.5.2 Implantable Device

First implantable device was invented in 1958, and since then numerous companies and researchers have dedicated themselves to the development and improvement of this field (Fig. 1.13a–d) [189–192]. The continuously emerging demand for implantable device is related with the rise of aging population in major developed countries. According to the research from Freedonia group, the market scale of implantable device in America is increased with a rate of 7.7% and reached 52 billion dollars in 2005 [193], and the largest share of market is taken by implantable rectification equipment such as artificial joints and bones. Other types of implantable device include cardiovascular related apparatus (cardiac pacemaker and intravascular stent), nerve stimulator and drug delivery carrier. Though many implantable devices of medical intention are aimed at aging population, more and more newly emerged products are targeting at the body shaping market of young people. Medical implantable device that is totally or partly prepared by micromachining has been well commercialized and applied in treatment to different diseases. For example, acceleration sensor is equipped to cardiac pacemaker and defibrillator to sense body location and movement. Electronic instruments of this type require

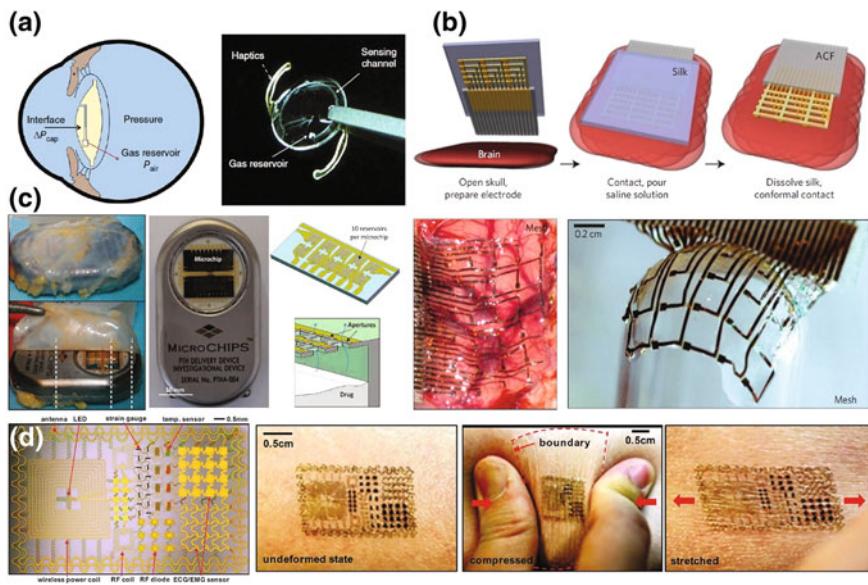


Fig. 1.13 Implantable microchip device. **a** An implantable microchip for intraocular pressure monitoring. (Reprinted with permission from Ref. [189]). **b** Ultrathin conformal bio-integrated electronics for brain monitoring (Reprinted with permission from Ref. [190]). **c** Wirelessly controlled drug delivery microchip (Reprinted with permission from Ref. [191]). **d** Multifunctional electronics with physical properties matched to the epidermis (Reprinted with permission from Ref. [192])

built-in power source, which occupies large space and may do harm to patient's health if leakage happened. Recently developed wireless charging system can avoid the problems encountered by built-in power design and extend the validity period of device. But the disadvantage is requirement of external supporting equipment.

Material choice of implantable device is strictly limited by several standards. Firstly, the candidate must be biocompatible which causes no toxic, allergic, inflammatory or immune reaction of the body. Secondly, stable must the material maintain in sterilization treatment such as heating, chemical reagents washing, radiation and high pressure. These treatments can't harm the normal functionality of device. Finally, the chosen material should possess corresponding electronic or mechanical properties to realize full device functions.

Future development of implantable device rests with the integration of monitoring and treatment in one device to achieve a closed loop from diagnosis to therapy according to the personalized situation of patients. Besides, through online information interchange, doctors at remote terminals can receive the monitoring data and give medical intervention in time.

1.5.3 Smart Mobile Device

Another direction for microfluidic device development is to combine microchip portal detection with the data analysis, management and transportation of smart phone and establish a mobile analytical platform. This concept is also called MS² (Mobile sensing based on microfluidics and smartphone) [194]. Actually, various built-in sensors as well as external detectors have been used to acquire health data of user with smart phone APP. The combination of microfluidic device with smart phone greatly expands the data acquisition ability of mobile phone (Fig. 1.14a-c) [195–197]. At the same time, features of immediate information processing and transportation of smart phone makes detection results no longer restricted to temporal and regional factors and instant feedback available. Common characters shared by two devices such as miniaturized structures, portability and simple operation enable the successful combination and establishment of mobile analytical platform. Smart mobile sensing can realize remote in-field monitoring, help improve family health care and provide medical aids to resource limited areas. Its application has involved fields of food quality monitor, regular health examination and disease diagnosis.

As a pioneer of this discipline, Martinez and coworkers from Harvard firstly connected paper based microchip to a smart phone and applied the device to glucose and protein detection in artificial urine sample at 2008 [198]. Then in 2009, Lu and colleagues designed an immune test device assembled by PDMS chip and smart phone [199]. The combination of microfluidics with mobile phone not only provides a simple solution for portable detection, but also makes remote medical diagnosis a reality.

However, the biggest limit of applying mobile sensing device to regular physical examination now is that mobile device doesn't reflect an irreplaceable detecting

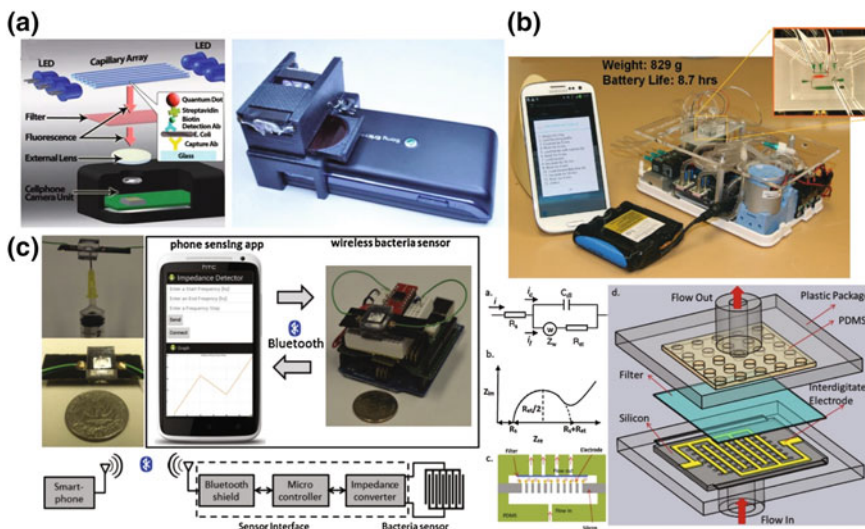


Fig. 1.14 Smart phone combined microchip device. **a** Quantum dots based assay for *E. coli* detection on cell phone. (Reprinted with permission from Ref. [195]). **b** Smartphone controlled handheld microfluidic liquid handling system (Reprinted with permission from Ref. [196]). **c** Smartphone based portable bacteria pre-concentrating microfluidic sensor (Reprinted with permission from Ref. [197])

feature, and medical workers prefer to use conventional methods. Moreover, the sampling of MS² equipment requires additional pipette or flow pump, which largely affects the portability and easy operation. In the future, the human-device interface needs further optimization by integrating sample inlet directly on device, such as installing blood taking needle or swab. Certain protection must be installed to prevent sample contamination in a complicated working environment. And owing to poor telecommunication and infrastructures in some regions, detection results must be stored in device for a certain period. This limitation can be overcome by increasing battery capacity and endurance.

Only after mass production and wide acceptance by consumers, can a value of technology be reflected. Smart mobile sensing is an emerging territory of microfluidics and we will wait and see what a role it can play in future.

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