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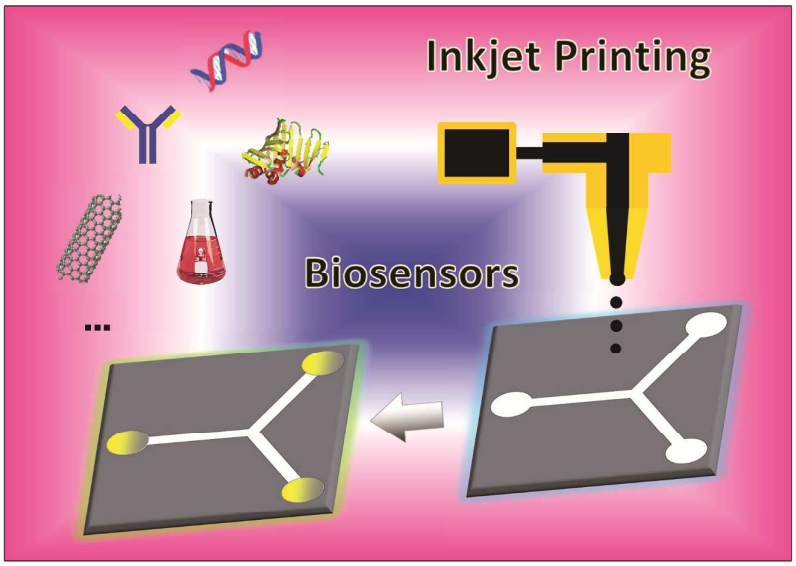

Inkjet printing is emerging at the forefront of biosensor fabrication technologies.

Journal Name

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Inkjet printing for biosensor fabrication: combining chemistry and technology for advanced manufacturing

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Cite this: DOI: 10.1039/x0xx00000x

Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

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Inkjet printing is emerging at the forefront of biosensor fabrication technologies. Parallel advances in both ink chemistry and printers have led to a biosensor manufacturing approach that is simple, rapid, flexible, high resolution, low cost, efficient for mass production, and extends the capabilities of devices beyond other manufacturing technologies. Here we review for the first time the factors behind successful inkjet biosensor fabrication, including printers, inks, patterning methods, and matrix types. We discuss technical considerations that are important when moving beyond theoretical knowledge to practical implementation. We also highlight significant advances in biosensor functionality that have been realised through inkjet printing. Finally, we consider future possibilities for biosensors enabled by this novel combination of chemistry and technology.

Introduction

Inkjet printing is a non-impact printing technology that deposits ink in the familiar patterned array known as the dot matrix. It is based on digitally controlled ejection of fluid drops from a small aperture directly to a pre-specified position.[1] The original idea of an inkjet is attributed to Lord Rayleigh in 1878, who proposed a liquid jet of constant radius able to fall vertically under gravity.[2] As the liquid length increases and reaches a critical value, the jet loses its cylindrical shape and decomposes into a stream of droplets (Fig. 1). Lord Rayleigh's idea was applied first to a recording device in 1930, but it was not until Rune Elmqvist patented the first commercial inkjet recorder in 1951[3] that the idea was more widely adopted.

In 1961, Sweet from Stanford University developed the first continuous inkjet.[4] This type of inkjet applied a pressure wave pattern onto the ink stream that could be broken into droplets of uniform size and spacing. Later, extensive development by IBM allowed the application of this continuous inkjet technology to computer printers.[5] In the mid-1970s, Zoltan invented drop-on-demand (DOD) piezoelectric inkjet printing.[6] It differed from the continuous inkjet by ejecting ink droplets only when needed. Modern-style inkjet printers were first displayed in 1979, by Canon and Hewlett-Packard (HP) who developed two similar types of inkjet printers. These used pressure generated from the growth and collapsing of water droplets to control ejection. The former was called a bubble jet[7] and the latter was named as *Thinkjet*[8].

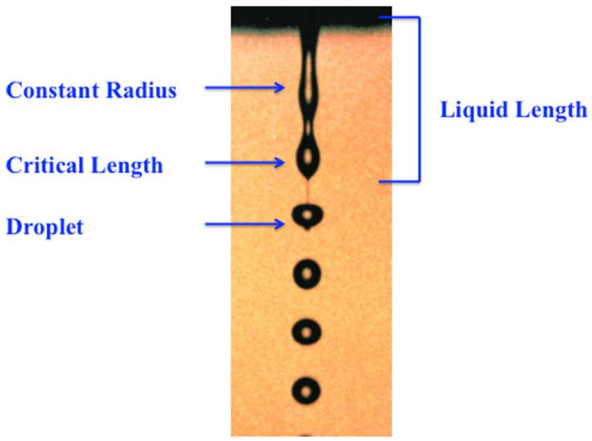

Fig. 1 Lord Rayleigh's idea of instability of jets. From Ref. [9]. Copyright 2001 Society of Photo Optical Instrumentation Engineers.

While the best-known application for inkjet printing is photos and graphics, in 1985 the use of inkjet printers to print other functional materials was first described.[10] Nowadays, the uses of inkjet printing

are diverse and include large-scale high-end products such as organic thin-film transistors,[11] single-crystal films for microelectronics,[12] light-emitting diodes,[13] and solar cells[14]. Indeed, inkjet printing is no longer limited to two dimensional (2D) structures, but is emerging for 3D structures, as exemplified by ceramic scaffolds,[15] drug formulations,[16] and live tissues[17].

One particularly interesting and highly beneficial application of inkjet printing is the fabrication of sensors and biosensors and this is the focus of our review. While these sensors can be fabricated using other technologies, a topic reviewed by Gonzalez-Macia *et al.*[18], the use of inkjet printing provides distinct advantages over these other fabrication technologies. The method is extremely versatile for development of prototype biosensors, and enables rapid manufacturing *via* high pattern precision and resolution. The applications of such technology are numerous, and have been reviewed by Gonzalez-Macia *et al.*[18] and Komuro *et al.*[19]. Di Risio and Yan[20] have also provided a detailed review on dispensing biomolecules on bioactive paper. Here, we focus on the manufacturing technology itself, which is critical for understanding the limitations and advantages when applied to biosensor fabrication.

Our review comprehensively describes the major types of inkjet printers, the basic ink components, the functional molecules that can be incorporated into ink formulations, and the relationship between ink formulation, ink rheological properties and their compatibility with print heads. In addition, we address strategies for inkjet patterning of reaction chambers, and discuss critical parameters for this technique, as well as post-production processes. Our review serves as a detailed primer for general chemists interested in modern fabrication technologies for biosensing and other applications. We also highlight some outstanding functionality developed using inkjet printing that opens new frontiers for biosensor technologies. We conclude by critically considering future perspectives and developments for inkjet printing and biosensor development.

Inkjet printers

The machines to precisely form ink droplets have been the subject of intense commercial development.[7, 8] This has led to two main modes of operation, continuous and drop-on-demand. Further subdivisions for each operation mode are listed in Figure 2.

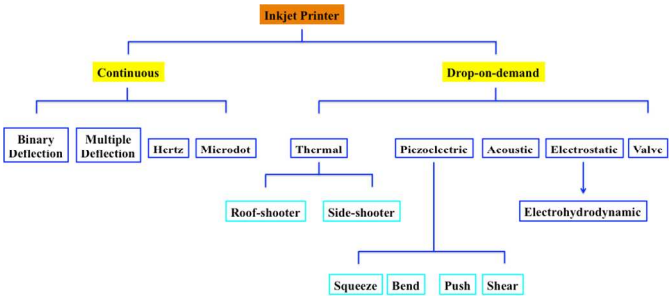

Fig. 2 Categories of inkjet printers.

Continuous inkjet printer

In a continuous inkjet printer, the creation of ink droplets is constant, and this is controlled by a high-pressure pump vibrating the nozzle with a piezoelectric crystal. The generated ink droplets are selectively charged *via* signals from the printer. Charged droplets are deflected into a gutter for recirculation, while the uncharged droplets are ejected onto the matrix to form an image (Fig. 3).[1] The droplets generated are usually twice the size in diameter than the printing orifice; 150 μm is the typical drop size, but it can be as small as 20 μm.[9] The advantage of the continuous inkjet printer is that it performs printing at high speed, and thus is very useful in an industrial environment. The printing nozzle is not easily clogged, as the ink droplets are generated continuously and volatile inks are used to allow rapid drying. However, the resolution can be reduced due to the high-speed of printing. Other disadvantages of the continuous inkjet printer include: (1) inks are restricted to those that can be charged; and (2) the printer is relatively expensive because of the requirement for drop selection, a recycling system, and a generally high maintenance cost.

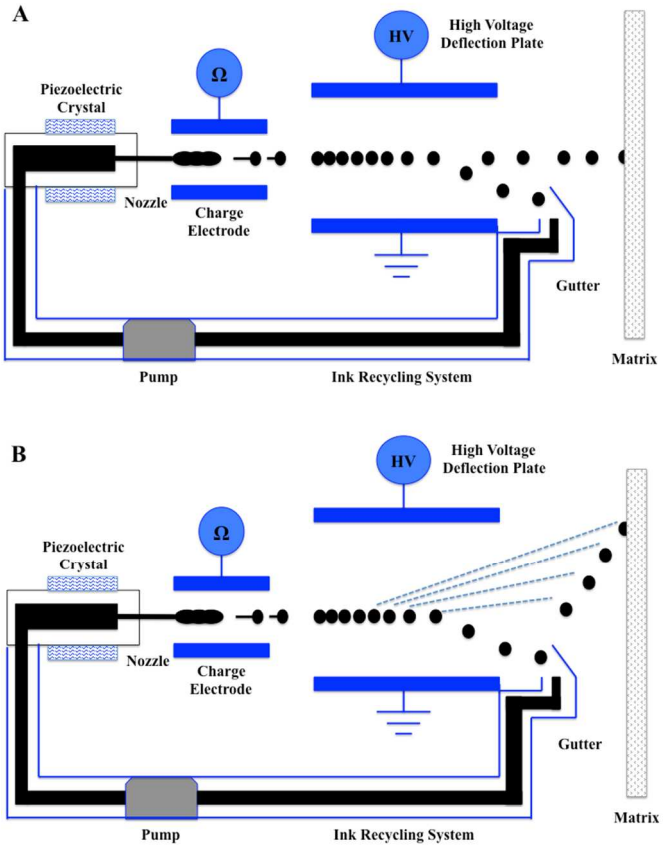

Fig. 3 Continuous inkjet printer systems. Ink droplets are constantly ejected, and the ejection is pumped by a piezoelectric crystal. The ink droplets are selectively charged *via* the printing signals. The charged droplets are deflected into a gutter for recirculation when passing through an electric field, while the uncharged droplets are ejected onto the matrix to form an image. A: In a binary deflection system, the uncharged droplets are printed onto the matrix and a single nozzle can only print at one dot position; B: In a multiple deflection system, the charged droplets are deflected onto the matrix. Multiple dots are deposited per nozzle.

In terms of the drop deflection method, the continuous inkjet printer can be classified into four systems (Fig. 2): binary deflection, multiple deflection, hertz, and microdot. For the binary deflection system, the uncharged droplets are printed onto the matrix and a

single nozzle can only print at one dot position (Fig. 3A).[1, 21] In contrast, in the multiple deflection system, the charged droplets are deflected onto the matrix; this allows multiple dot positions per nozzle (Fig. 3B).[1, 22] By virtue of this multiple deposition, the multiple deflection system prints faster than the binary deflection system. The hertz system is a modified version of the binary deflection inkjet system and it has improved color printing capability of the binary system. The number of ink droplets deposited is controlled through the volume of ink in each pixel in the hertz system. Consequently, the color density can be manipulated to achieve the desired gray tone.[23, 24] The microdot ejects large and small diameter droplets from the nozzle, however, only the droplets with small diameter are entered into the electrical field for selective ejection.[25] This is remarkable, because small ink droplets can be produced without reducing the diameter of the inkjet nozzle.[25]

Drop-on-demand inkjet printer

In contrast to the continuous inkjet printer, the drop-on-demand (DOD) inkjet printer ejects the ink only when it is required. The DOD eliminates the complex droplet charging, deflection and recycling system required for the continuous inkjet printer, and allows smaller drop size generation and higher placement accuracy. The ejected drop size approximates the diameter of the orifice, and less than 20 µm droplets can be achieved.[9, 26] The DOD printer relies on a pressure pulse created to form ink droplets. The method used to generate this pressure pulse defines the primary subclasses of the DOD printer, namely thermal,[24, 27] piezoelectric,[6, 28, 29] acoustic,[30, 31] electrostatic,[32] electrohydrodynamic (EHD),[33-35] and valve[36] methods. The first two are dominant in modern inkjet printing, EHD is becoming prominent, and the others are still in the developmental stage.

Thermal inkjet printer

The development of the thermal inkjet printer was inspired by the natural process of water boiling to form water bubbles.[7] In this technology, the ink in the ink chamber is rapidly heated up to a high temperature (350 to 400 °C) to vaporize. The vaporization promptly creates a bubble at the surface of a heater (resistor), causing a pressure pulse to push the ink droplets out through the nozzle. As the ink droplets are ejected, the vapor bubble collapses, which generates a force to refill the ink (Fig. 4A).[37] The entire procedure is fast, taking less than 10 microseconds. Depending on the location between the nozzle and the heater, the thermal DOD printer can adopt either "roof-shooter" or "side-shooter" configurations. The "roof-shooter" (Fig. 4B) has the nozzle located on the top of the heater, while the nozzle is located nearby the heater in the "side-shooter" (Fig. 4C).[1] The thermal inkjet printer offers high nozzle density and generates small ink volume (150 to 200 picoliters, pl, 10^{-12} of a liter), however, the ink chemistry is limited to vaporizable and thermally stable inks.

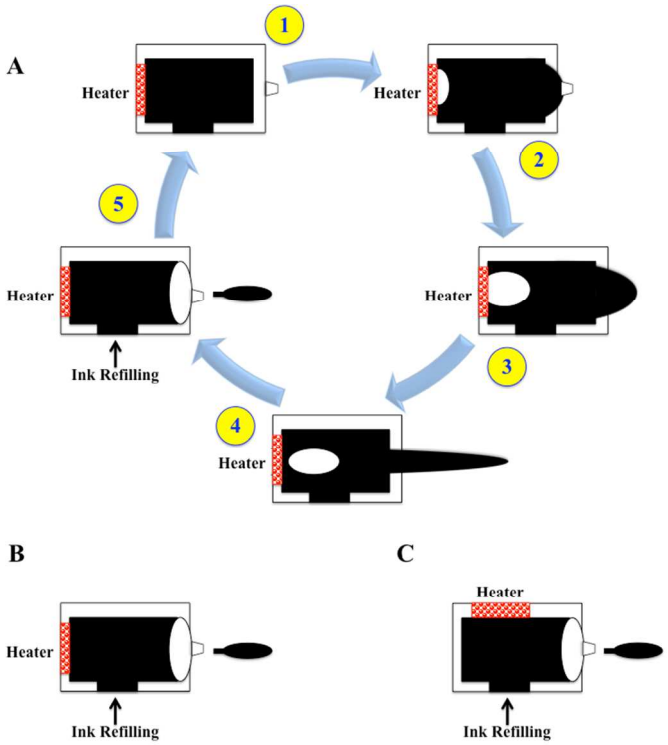

Fig. 4 Drop-on-demand thermal inkjet printer. A: Mechanism. Ink is rapidly heated to a high temperature to vaporize which creates a bubble at the surface of a heater causing a pressure pulse that exudes ink droplets through the nozzle. The vapor bubble collapses, as the ink droplets are ejected, thereby generating a force to refill the ink; B: Thermal "roof-shooter" configuration; C: Thermal "side-shooter" configuration.

Piezoelectric inkjet printer

Unlike the thermal inkjet printer, that uses the expanding and collapsing of ink bubbles by heating to control the ejection, the piezoelectric inkjet printer applies a piezo-ceramic plate to create ink droplets. A thin diaphragm is bonded to the piezo-ceramic plate to prevent unintended interactions between the inks and the plate. The piezo-ceramic plate deforms in response to an electric impulse. This generates a pressure wave that causes the ink to be ejected out from the nozzle. On the removal of the electric pulse, the ink is replenished as the piezo-ceramic plate returns to its normal shape.[1, 37] Since the ink is not heated to a high temperature (as in the thermal inkjet printer) the piezoelectric inkjet printer accepts a wider range of inks. The print head has a longer life since it is not subject to heat damage. The drop volume is around 150 pl, which is comparable to the thermal inkjet printer.[38] However, the cost of the print head and the associated software (that directs the head to apply certain droplets of ink per dot) is considerable.

The piezoelectric inkjet printer can be classified into squeeze, bend, push and shear mode based on the distortion of the piezo-ceramic plate. The squeeze mode (Fig. 5A) comprises a radially polarized piezo-ceramic tube surrounding the nozzle.[1] In both the bend (Fig. 5B) and push modes (Fig. 5C), the directions of the electric field and piezo-ceramic plate deformation are in parallel.[29, 39] Whereas in the shear mode these two directions are perpendicular to each other (Fig. 5D).[1] In all the modes, the voltage strength, the pulse duration and the orifice diameter influence the size of the ink droplets.

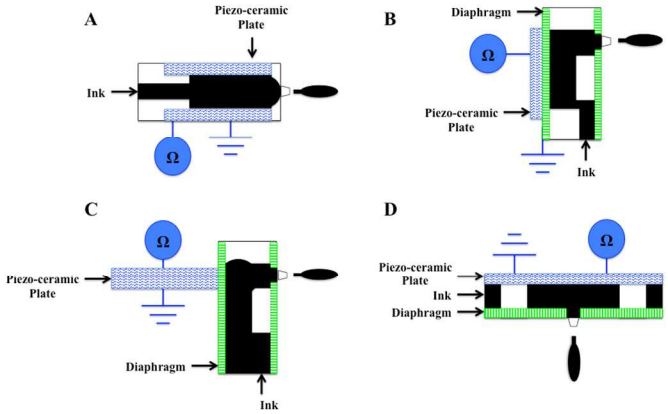

Fig. 5 Drop-on-demand piezoelectric inkjet printer. The piezoelectric inkjet printer applies a piezo-ceramic plate to create ink droplets. A thin diaphragm is bonded to the piezo-ceramic plate to prevent unintended interactions between the inks and the plate. The piezo-ceramic plate deforms in response to an electric impulse to generate a pressure wave to eject the ink. The ink is replenished on the removal of the electric pulse. At the same time, the piezo-ceramic plate returns to its normal shape. A: squeeze mode; B: bend mode; C: push mode; D: shear mode.

Other DOD inkjet printers

Other DOD inkjet printers are advances on the above technologies. For instance, instead of applying the electric pulse to deform a piezo-ceramic plate, the electrostatic inkjet printer (Fig. 6A) dispenses the ink droplets by directly modulating the electric field.[40] When the stream of ink passes through the electric field, the field imparts charges on the ink droplets. The jetting position of ink on the matrix can be controlled by varying the electrical potential applied on the plate. This allows deposition of droplets much smaller than the orifice diameter, producing finer droplets than the piezoelectric inkjet printer.[41]

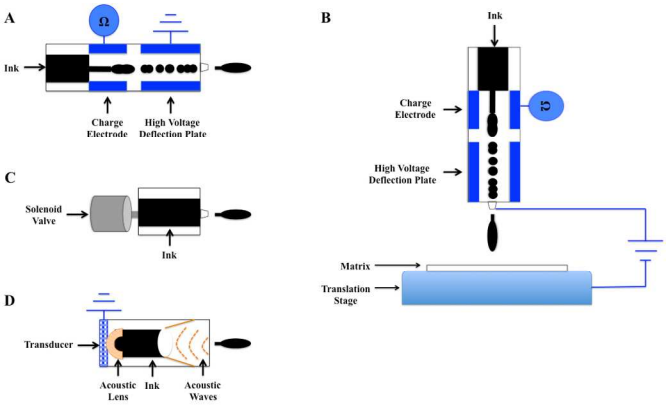

Fig. 6 Other drop-on-demand inkjet printers. A: electrostatic inkjet printer; B: electrohydrodynamic inkjet printer; C: valve inkjet printer; D: acoustic inkjet printer.

The electrohydrodynamic (EHD) inkjet printer (Fig. 6B) is an advance of the electrostatic inkjet printer in that printing is no longer controlled only by the print head but is synergistically manipulated *via* both the nozzle and a translation stage, both of which are connected to a voltage. The electric field generated between them creates an electrohydrodynamic phenomenon that induces ink ejection to the matrix.[35] A unique feature of the EHD inkjet printer is that the droplets ejected are from outside rather than from within the nozzle.[42] To do this, the EHD inkjet printer forms a droplet that is attached to the nozzle, the droplet then concentrates and jets out by overcoming the surface tension under electric field modulation.[42] This dual-controlled mechanism and special jetting mechanism enable the EHD inkjet printer to achieve nano-sized spots.[34] However, the ink application range for the electrostatic inkjet printer and the EHD inkjet printer is much narrower, since only conductive inks can be applied.

The valve inkjet printer (Fig. 6C) uses solenoid valves to control ink ejection. Resolution in this system is poor compared to the thermal, piezoelectric, electrostatic, and EHD inkjet printers, with a minimum drop size of 500 µm.[43, 44] In comparison to all the other DOD inkjet printers, the acoustic inkjet printer (Fig. 6D) does not have a nozzle. This inkjet printer adopts a high-frequency transducer to the back of an acoustic lens, which launches acoustic waves through the lens. By focusing the acoustic energy from the waves, this induces a pressure wave to expel the ink from the surface of the ink chamber.[45] The advantage of not having an orifice is the elimination of nozzle clogging, a common problem in the thermal and piezoelectric inkjet printers.

Inkjet printing inks

The unique chemistry of individual inks is indispensible to the inkjet printing system. This is because the ink properties not only dictate the printing quality but also determine the characteristics of the drop ejection and the reliability of the printing system.[1] The inks contain base and colorants, together with some additives, as well as components that endow biosensor functionality (*e.g.* signalling molecules), which are described in the subsequent sections. The base acts as the liquid carrier of the colorants, which enables them to bind to the matrix after printing.[46] Both the base and the colorants have subdivisions and will be described in details.

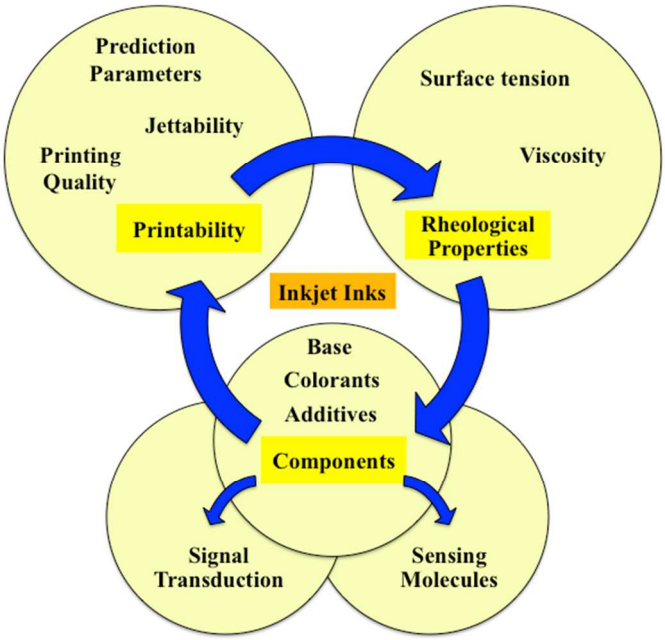

Fig. 7 Relationship between ink formulation, ink rheological properties and their compatibility with the print heads.

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Table 1 Typical fluidic parameters for ink drop ejection from the thermal and piezoelectric inkjet print heads

Dimensionless Groupings of Physical Constants	Equations	Parameters and Units	Ranges	References
Reynolds Number	$Re = \frac{\rho v L}{\eta}$	• ρ = Density of the fluid (kg/m³) • v = Velocity (m/s) • L = Characteristic linear dimension (travelled length of the fluid) (m) • η = Dynamic viscosity of the fluid (Pa·s or N·s/m² or kg/(m·s)) • γ = Surface tension (N/m)	50-500	47, 48
Weber Number	$We = \frac{v^2 \rho L}{\gamma}$		20-300	48, 49
Ohnesorge Number	$Oh = \frac{\sqrt{We}}{Re} = \frac{\eta}{\sqrt{\gamma \rho L}}$		NA	50
Z	$Z = \frac{1}{Oh}$		10 > Z > 1 for stable drop formation	51, 52

NA= not applicable

Base

The base is usually divided into four groups: aqueous-based, non-aqueous-based, phase-change and reactive. The main component of the liquid in the aqueous-based base is the ink. The non-aqueous-based base contains several organic solvents as well as the ink.[1, 46] The drying mechanism for both the aqueous-based and non-aqueous-based bases is that the ink evaporates and penetrates to the porous matrix simultaneously.[1] However, the image quality can be poor, because the ink tends to diffuse. Rather than being liquid at the ambient temperature, the phase-change base is solid at room temperature but melts to liquid while jetting and solidifies immediately once it reaches the matrix (a typical example is wax-like ink). Conventional phase-change inks consist of a wax containing ink binder, melting point between 90 °C to 150 °C, tackifiers, adhesion promoters, and additives.[53]).[1, 54] This property can overcome the image quality issue from the other two bases, as the phase-change base does not diffuse on the matrix. The aqueous, non-aqueous and phase-change bases can produce acceptable images when ejected upon a porous or coated matrix, however, they do not perform well upon non-porous matrices due to poor adhesion. The reactive base solves this problem by remaining as stable liquid until it is cured by UV irradiation. The curing triggers the liquid to undergo polymerization, leading to more compact adhesion to the matrix.[46] Thus similar to the phase-change base, the reactive base has less dispersion of the ink. However, the ink coverage on the matrix is correspondingly low, resulting in a higher ink consumption. Despite this, the reactive base is non-volatile and does not contain organic solvents, which is less toxic for the environment.[55]

Colorants

The colors produced in inkjet printing are due to the addition of colorants in the ink. The colorants are either dyes (organic or polymeric) or pigments.[1] The dyes are soluble and exist as individual molecules in the ink, while the pigments are insoluble and tend to form clusters. This chemical property distinguishes the dyes and pigments in color performance. The dye-containing ink is more stable than the pigment-containing ink, because the pigments sometimes aggregate to each other.[46] The aggregation will influence the ink flow and supply systems and even clog the print head. To address these problems, additives (*e.g.* surfactants, dispersants, polymers) are used to stabilize the pigments *via* electrostatic and/or steric mechanisms. For example, the anionic surfactant sodium dodecyl sulfate (SDS) is usually mixed with carbon black (hydrophobic). The imparted negative charge from the SDS prevents the carbon black pigment from aggregating, thus stabilizing the ink.[46] Although pigment-containing ink is relatively unstable, it is advantageous in terms of light fastness and humidity stability. This is because clustering properties of the pigments provide greater resistance towards these environmental impacts.

Additives

Apart from base and colorants, additives are other important ink constituents. Additives stabilize or adjust the property of the ink for printing and storage. The main types of additives include surfactants, viscosity modifiers, dispersants, humectants, biocides, and chelating agents.

To achieve good printing quality, ink viscosity and surface tension are the two most important rheological parameters (Fig. 7).[56, 57] Low viscosity ink disperses quickly on the matrix leading to poor image quality. Viscosity modifiers, such as glycerol, ethylene glycol, poly(vinyl alcohol), and sodium carboxymethyl cellulose can be used to regulate the viscosity.[40, 58, 59] High surface tension can prevent jetting out from the print head and cause it to clog, while low surface tension causes incorrect ink release, so that it streams out of nozzle or form unstable drops. For this reason, surfactants are usually applied to modulate surface tension (*e.g.* anionic surfactants such as Sodium dodecyl sulfate; cationic surfactants such as Cetyl trimethyl ammonium bromide; or non-ionic surfactants such as Triton X-100; zwitterionic surfactant, betaine).[60] Other reagents can alter multiple properties, for example, ethanol (a co-solvent) can

lower the surface tension while facilitating the sample solution wettability, and it also solubilizes insoluble pigments.[61-63]

Dispersants contain two classes: surfactants and polymers. They stabilize the pigments from aggregation by imparting charges *via* electrostatic and/or steric hindrance.[46] Ink formulation with good dispersion stability is critical for conductive inks (see conductive molecules section) and affects the function of the resulting biosensor. Woo *et al.*[64] reported that silver ink with stable dispersion exhibited low electrical resistivity due to decreased inter-particle junctions. Humectants (*e.g.* glycerol or glycerine) are mainly used to control or limit the evaporation of the inks. They act as hygroscopic agents during printing or in the idle position of printer to prevent clogging of print heads.[34, 65, 66] In order to control biological growth, biocides are also included in the ink formulation. Similarly, chelating agents (*e.g.* Ethylenediaminetetraacetic acid, EDTA) also have antimicrobial growth properties and can chelate unwanted trace metals from mixtures of dyes in the ink formulation.[40, 58, 67]

Importantly, the choice of additives must include considerations of compatibility. For example, ethanol may not be compatible with some biomolecules such as proteins. In this case, sugars (*e.g.* glucose, sucrose) can prevent biomolecules from denaturing and dehydrating during printing, as the sugar can form a rigid crystal to support the 3D structure of the proteins.[68] Alternatively, a carrier protein such as bovine serum albumin (BSA) can be included to stabilize proteins and minimize any non-specific adsorptions onto the ink chamber.[69]

Critical parameters for ink printability

The final ink formulation must be a collection of carefully titrated compatible components with properties that are (1) in lieu with the printable rheological properties of the printer, and (2) suitable to the chosen print head, leading to formation of stable droplets with good jettability. For (1) the theoretical printability can be calculated from three physical constants, namely the Reynolds number, Weber number, and Ohnesorge number (Table 1). In particular, the Z parameter (the inverse of the Ohnesorge number) is commonly used to indicate printability, where a Z value between 1 and 10 is expected to generate stable drop formation.[52] In addition, (2) is paramount, as the print head greatly affects printing quality (outside of interactions between the ink and the printing matrix). Incompatibilities can lead to an error in deposition precision, such as satellite droplets problem (Fig. 8). Several reports suggest including polymers in the ink to minimize the satellite droplet formation,[70-73] since polymers maintain attachment in the falling droplet.[72] Similarly, if the surface tension of the ink formulation is low, the ink can spread as a thin layer on the nozzle plate, causing faceplate wetting. Subsequent solidification after evaporation affects the trajectory of droplets or even inhibits jetting,[46] but can be prevented by addition of a surfactant to increase ink surface tension. Another type of nozzle clogging can occur after idle use, known as the "First drop problem".[74] Here, evaporation at the nozzle causes local changes in the ink composition and rheological properties,[66] and addition of a humectant or the usage of less volatile solvents can mitigate this problem.

Additional parameters to consider include the diameter of the nozzle, jetting voltage, stand-off distance, and humidity. The nozzle diameter controls the drop size deposited, and thus the printing resolution. The jetting voltage impacts the speed of the droplet firing; its adjustment can be manipulated according to the rheological properties of the ink. The stand-off distance (the distance between the nozzle and the matrix) affects the formation of the satellite droplets (Fig. 8), and printing accuracy and resolution.[75] Finally, a suitable and constant relative humidity maintains the stability and activity of the molecules (*e.g.* biomolecules and polymers), and a stable printing process (*e.g.* ink evaporation).[76]

In summary, the ink formulation, ink rheological properties and their compatibility with the print heads are closely inter-related. Modulation of these parameters is a trial and error process and is critical for successful inkjet printing.

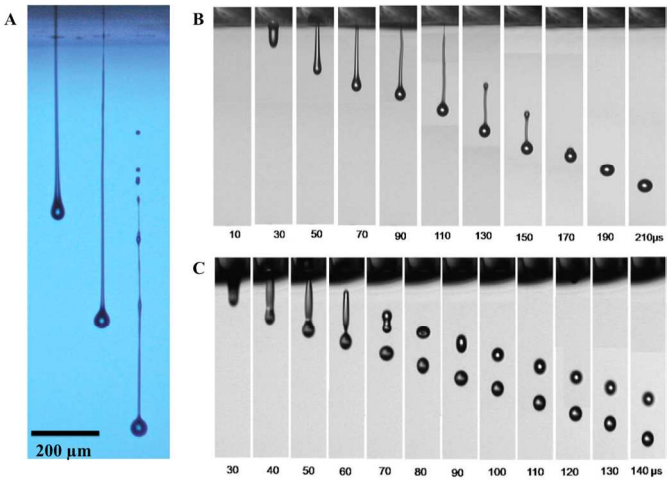

Fig. 8 Satellite droplets of inkjet printing. A: a high-speed photographic image showing three drops ejected from a DOD printer at different stages of drop formation. From left to right: the drop forms from a single ejected liquid column that rapidly forms a leading droplet followed by a ligament. The tail breaks up into a trail of satellite droplets behind the leading droplet. © IOP Publishing. Reproduced with permission from Ref. [77]. All rights reserved. B: satellite droplets formation with Z value of 3.57; C: satellite droplets formation with Z value of 17.32. The satellite droplets retract to the leading droplet in B (when Z value lies between 1 and 10) but not in C (when Z value lies outside of the range of 1 and 10). Reprinted with permission from Ref. [75]. Copyright 2009 American Chemical Society.

Inkjet printed biosensors

The purpose of a biosensor is to detect an analyte, and inkjet printed versions of these devices are useful in various fields ranging from industrial to clinical.[78, 79] To achieve analyte detection, a biosensor critically requires (1) sensing molecules that interact with the analyte and (2) a transducer (physicochemical detector) to transform the interaction into a measurable signal (Fig. 9). In addition, contemporary biosensors can also contain a series of interconnected zones for sophisticated interactions between components. Inkjet printing provides a rapid, inexpensive, and convenient method to deposit some or all of these components with high precision. Partially printed devices only use the inkjet printer as one application in the sensor fabrication process,[80-82] however, the multiple cartridges inherent in coloured inkjet printers provide the opportunity to rapidly and precisely deposit multiple

components at once, and fully inkjet printed devices have been reported[63, 83-85] (also see Table 2).

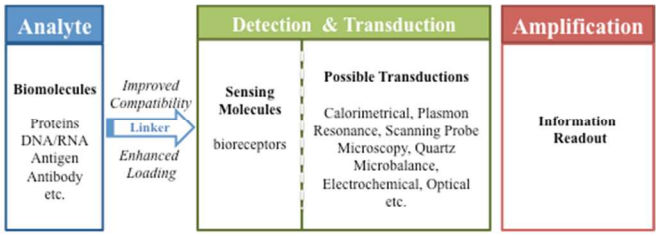

Fig. 9 Mechanism and components of biosensing systems.

Inkjet deposition of sensing and transducing molecules

The sensing molecules used in biosensors can be enzymes, antibodies, or proteins, hormones, nucleic acids, and even micro-organisms or whole cells. They can also include synthetic components with biomimetic properties. Some of the considerations for incorporating biomolecules or conductive molecules as ink components are described in the next sections.

Transduction of the analyte/sensing molecule interaction can occur *via* any of the wide variety of systems available for biosensors, such as electrochemical, optical, potentiometric, amperometric, calorimetric, enthalpimetric, impedance spectroscopy (IS), piezoelectric detection, surface plasmon resonance (SPR), surface enhanced raman spectroscopy (SERS), scanning probe microscopy (SPM), or quartz crystal microbalance (QCM), *etc.* (Fig. 9).[86] The choice of the transduction mechanism depends on the application field, but the most widely used measurements for inkjet printed biosensors have historically been electrochemical and optical, yet optical-based biosensors are advantageous of being more point-of-care inclined.

An example electrochemical biosensor is the glucose oxidase sensor described in the whole surface patterning section below (see Fig. 10A), and further examples are provided in Table 2 (#2-5). Electrochemical techniques can be amperometric, conductometric, voltammetric or potentiometric. The most versatile technique is voltammetry, since it allows both current and potential measurements for a short response of the system, with possibilities for multi-component detection.[87]

Optical techniques collect photon measurements of the analyte/sensing molecule interaction, rather than electrons. These can be based on luminescence, fluorescence or color change, and can be measured by absorbance, or reflectance of fluorescence emissions in the ultraviolet (UV), visible, or near-infrared (NIR) regions of the light spectrum.[88] Colorimetric measurements are most popular for point-of-care biosensors, and examples constructed using inkjet printing are provided in the Inkjet patterning sections below (Fig. 10B – 14A).

Biomolecules

Examples of ink-jet printed enzymes include the glucose oxidase biosensor (Fig. 10A) and β-galactopyranoside/β-galactosidase in a bi-directional lateral flow dipstick (Fig. 10B). In addition, several examples of ink-jet printed antibodies are described in the inkjet patterning sections below (Fig. 11B, 12A, 14A). For inkjet printing of these and other biomolecules, the manufacturing technique and the intrinsic molecular properties are important considerations during formulation of the inkjet ink, as are the quantity of material available and its stability.

A major issue for these biomolecules during inkjet printing is their non-specific adsorption onto the ink chamber. This quantity loss can have an impact on the printing quality and cost. While antibodies, hormones, proteins, and enzymes can be expressed, the time it takes to accumulate large quantities of these molecules is much longer than with nucleic acids and cells which can be amplified relatively easily. Delehanty and Ligler[69] found that the addition of BSA could minimize protein loss in the ink tank due to adsorption. Interestingly, the BSA additive also optimized the spot uniformity.

Another major issue for biomolecules is the maintaining of their stability during printing. Most biomolecules, except certain nucleic acids, tolerate heat in the range between 40 • to 80 •.[89-92] Therefore, the piezoelectric inkjet printer compares favorably with the thermal inkjet printer, since the piezoelectric printer does not employ heating mechanisms for ejection. However, the practical reality of these expected conventions needs to be assessed on a case-by-case basis. Viravaidya-Pasuwat *et al.*[93] modified a commercial thermal inkjet printer (Cannon IP 2700) to print BSA (together with a red dye for visualization) onto a nitrocellulose slide. The BSA could be repeatedly printed as a 1 mm spot 20 times on one nitrocellulose slide without clogging. Roda *et al.*[94] also showed that there was no denaturation of horseradish peroxidase (HRP) printed by a thermal inkjet printer (DeskJet 600). Whereas, Lonini *et al.*[95] found that the polyclonal rabbit anti-human IgG labeled with HRP caused clogging when printed by a thermal inkjet printer (HP Deskjet 5740) but the same protein could be printed by piezoelectric inkjet printing with no problems. Similarly, Setti *et al.*[40] measured a 15% loss of β-galactosidase activity after printing by a thermal inkjet printer (Olivetti Tecnost).

In addition to the thermal stress, another problem is the printing shear rate. For current DOD inkjet printers, the shear rate ranges from 2×10^4 to 2×10^6 s^{-1}, which may potentially destroy proteins.[96] Another stress, pointed out by Nishioka *et al.*[97] is the compression rate, a force exerted on the ejecting ink to improve resolution and to enhance penetration of the ink into the paper. They found that even an extremely low compression rate (2.56×10^4 $\mu m^3/\mu s$) caused damage to peroxidase. However, the addition of sugar (trehalose or glucose) significantly reduced the damage.

In summary, the non-specific adsorption and all the above mentioned tumultuous disturbances can affect inkjet printing of these biomolecules, yet using suitable additives (see additives section) can reduce or even eliminate these disturbances.

Conductive molecules

Conductive molecules used for inkjet printing of biosensors include nanoparticles, organometallic compounds, and conductive polymers (*e.g.* PEDOT/PSS in Fig. 10A; PVAm in Fig 10B; and CuO, Si, Ag, or TiO_2 nanoparticles, Table 2 # 3,10-12,31).[98] Other novel nanomaterials (*e.g.* carbon nanotubes and graphene) are also emerging as popular molecules for inkjet fabrication of biosensors.[99-103] In comparison to biomolecules, quantity is less of an issue during ink formulation than stability. In particular, the conductive molecules need to exist at a uniform dispersion state to prevent

agglomeration. Stabilization of these molecules relies on the addition of additives (*e.g.* dispersants and polymers, see additives section). Polymers are also used as additives to prevent satellite droplets (see critical parameters for ink printability section), and so the concentration of conductive polymer should be adjusted to avoid generating long strands of liquid. In addition, an inert environment is required for inkjet printing of conductive molecules, as these molecules are highly susceptible to ambient humidity and reactivity with oxygen. Moreover, the ejection velocity needs to be well controlled, as it affects the distribution of the dispensing conductive molecules.[104]

Advantage of using inkjet printing of sensing molecules

In comparison to the other dispensing methods (*e.g.* screen printing and microspotting), the dispensing of sensing molecules using inkjet printing is advantageous because: (1) printing is straightforward and inexpensive *via* cheap desktop printers; this is especially true for carbon nanotube deposition, where the conventional chemical vapour deposition method requires complex processes and sophisticated techniques[105]; (2) there is a low risk of contamination, since inkjet printing is a non-contact method; (3) minimal materials are wasted, particularly for DOD where the ink is only ejected as required; (4) multiple sensing molecules can be printed using a single device,[106] since inkjet printers usually have multiple ink cartridges and printing nozzles; (5) printers enable precise spatial control without any cross interference on a matrix;[107, 108] and (6) printing enables gradient creation, whereby different densities of inks can be placed in desired regions (also see inkjet patterning on superhydrophobic matrices section).[109-111]

Inkjet patterning

Unlike the dispensing of the sensing molecules, inkjet patterning is about dispensing certain reagents on a matrix to create a reaction surface, or to define liquid pathways as channels or zones for the sensing event(s). Inkjet patterning inherits all the merits of inkjet dispensing for sensing molecules, and in addition permits new functionalities, which is a benefit for simplicity and miniaturization of biosensors for point-of-care diagnostics.

Whole surface patterning

Whole surface patterning involves the inkjet printing a mono- or multiple-layers of material to cover the entire surface of a matrix to serve as a reaction phase. In some biosensors, the reaction surface that contains biomolecules is on the top layer. Setti *et al.*[58] fabricated an amperometric glucose biosensor prototype by a thermal inkjet printer. The thermal inkjet printer was applied to deposit two types of inks – electronic ink and biological ink. The electronic ink is composed of conductive polymer [poly(3,4-ethylenedioxythiophene/polystyrene sulfonic acid), PEDOT/PSS], which was printed onto an indium-tin-oxide (ITO) coated glass surface (Fig. 10A). The biological ink that contained glucose oxidase (GOD) with buffer and additives was then deposited onto the polymeric film. The printed polymer provided a good electron transfer surface for the redox reaction between the glucose and the GOD with no loss of enzyme activity.[58] By applying the same procedures, Setti *et al.*[112] fabricated an HRP-based amperometric hydrogen peroxide biosensor, which showed the same success but with a higher inkjet printing resolution (1500 × 1500 dot per inch, dpi). Based on Setti and co-worker's work, Yun *et al.*[113] fabricated a prototype of a bienzymatic (GOD and HRP glucose) biosensor using a piezoelectric inkjet printer (Dimatix Materials Printer DMP-2800). The bienzymatic glucose biosensor displayed a fast response time (<3 seconds). Also, their result showed that the inkjet printed conductive surface improved the current response approximately 1.5 times.

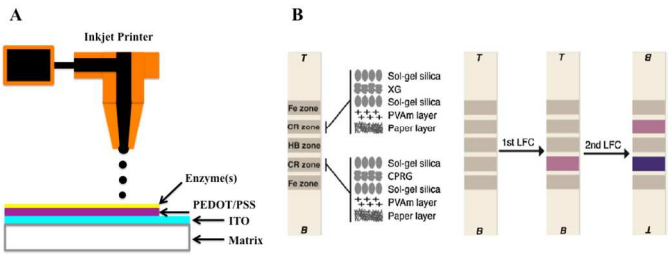

Fig. 10 Inkjet printer fabricated biosensors with surface patterning. A: schematic diagram of total inkjet printed GOD and/or HRP biosensor using whole surface patterning strategy. B: inkjet fabricated bi-directional lateral flow dipstick using whole surface patterning strategy *via* entrapment (T: top, B: bottom). From Ref. [114]. Copyright 2012 Springer-Verlag.

Entrapment

Although printing the biomolecule-containing surface as the top layer demonstrates effective enzymatic or electrochemical reactions, the shelf-life of this patterning is relatively short (<1 or 2 weeks) due to the instability of the biomolecules. A commonly-used surface patterning strategy is to entrap the sensing reagents between the other reagent layers, which retains the stability and activity of the biomolecules. Weng *et al.*[115] applied this strategy in printing a similar HRP-based amperometric biosensor to that of Setti.[112] The polypyrrole (PPy)/enzyme layer was sandwiched between a bottom PPy layer and a top ethyl cellulose (EC) layer. However, the resulting sensitivity was 0.5 times lower (0.25 μAM^{-1} cm^{-2}) than Setti's device (0.544 μAM^{-1} cm^{-2}).

The same patterning strategy was also conducive to lateral flow devices. Hossain and co-workers[114] built a bi-directional lateral flow dipstick using a piezoelectric inkjet printer (Fujifilm Dimatix 2800 N) for bacterial detection. The inkjet printer printed four layers: a capture polyvinylamine (PVAm) layer (on top of the matrix), a lower sol-gel-derived silica layer and a top silica layer between which embedded inkjet deposited sensing solutions (Fig. 10B). The dipstick assay was sensitive and could detect less than 10 cfu/mL of *E. coli* without culturing if immune-magnetic separation (a sample pre-concentration step) was used. The inkjet printing entrapped sensing reagents were stable, with no reagents denaturation occurring for at least two months at room temperature (25 ℃).Wang *et al.*[116] subsequently explored the function of each printing layer. They determined that the bottom layer isolated the entrapped sensing molecules from the cationic PVAm layer, preventing the potential inhibition of the sensing molecules, while the top layer protected the sensing molecules from proteolysis. The inkjet printed sol-gel (top and bottom layers) confined the sensing molecules in their initial place after lateral flow detection. A transmission electron microscopy (TEM) analysis indicated that the inkjet printed sol-gel material formed a thin film (with thickness of 35 ± 15 nm) that promoted rapid substrate transport and enhanced mechanical stability.[116] Such lateral flow dipsticks could also be adapted to detect other β-glucuronidase and β-galactosidase producing bacteria or to

other intracellular enzyme makers by simply varying the reaction reagents.[114] The same research group also successfully demonstrated the detection of neurotoxins and pesticides on lateral flow devices by using the same entrapment strategy.[117, 118]

Inkjet patterning as channels or zones

In contrast to whole surfacing patterning, inkjet printing can also be used to patterning reaction channels or zones. That is, rather than printing a homogenous surface, selective sections of the surface are printed with inks of varying properties, to define liquid passageways and sensing areas. A range of methods for patterning channels or zones have been reported including photolithography using photoresistors,[119-125] plotting with poly(dimethylsiloxane) (PDMS),[126] cutting,[127-129] wax printing,[130-137] wax dipping[138] or wax screen-printing,[139, 140] plasma treatment,[81, 141] marker pens,[142] flexographic printing,[143] and laser treatment[144]. Each of these methods has advantages and disadvantages. For instance, the physical barrier generated by photolithography lacks flexibility and is not resistant to bending and folding damage.[126] Although plotting with PDMS overcomes the flexibility issue, the topology of channels formed is not straight.[141] Similarly, while the plasma treatment improved the topological issue, it requires fabrication of masks for each channel pattern. Likewise, the flexographic printing requires two prints of polystyrene solution and different printing plates for each printing, but has issues with resistance to the low surface tensions of fluid.[143] Patterning using cutting or a marker pen is simple, rapid, and low cost, but the border of defined channels is not smooth. The printing of wax results in low resolutions. In particular, the wax tends to spread out when it melts, and thin reaction channels are hard to achieve. Finally, laser treatments can generate high resolution, but the channels patterned do not allow lateral flow of fluids—this requires extra coating (*e.g.* hydrophilic silica microparticles) to properly channel liquid flow.[144]

In contrast, an inkjet printed channel or zone can be produced rapidly and reproducibly, with high resolution and flexibility of design that is efficient for mass production. In general, inkjet patterning of reaction channels or zones on a porous matrix (usually hydrophilic) aligns with the principle of enclosing the hydrophilic portion of the matrix (*e.g.* cellulose membrane) by hydrophobic barriers. This can be achieved indirectly and directly.

Indirect inkjet patterning as channels or zones

The indirect approach to channel or zone patterning is to fully hydrophobize the matrix at the first step, and then apply a hydrophobic solvent to locally dissolve the hydrophobic cover, thus re-exposing the hydrophilic matrix as channels or zones in desired patterns. Abe *et al.*[63] built a multi-analyte chemical biosensor for the detection of pH, total protein (human serum albumin, HSA), and glucose simultaneously using a piezoelectric inkjet printer (PicoJet-2000). They applied toluene to the pre-printed hydrophobic cover (polystyrene treated filter paper) to generate reaction zones. When the sample solution was applied to the central loading area, it moved evenly toward the sensing areas (Fig. 11A). The hydrophobic ink penetrated deep through the porous matrix, forming a strong barrier that guided liquid smoothly without incurring leaking to the other channels.[63] Their second fabrication was a lateral flow device based on a sandwich assay (involving antibody-antibody interactions, Fig. 11B (ii)).[83] The lateral flow device required several consecutive sensing reagent deposition areas, necessitating a change in the printing order of the hydrophilic channels. In this case, the control and the test regions, along with the sensing reagents in these two regions were printed first, and then followed by implementing the other channels and the rest of the sensing reagents (Fig. 11B (i)). This was to prevent the spreading of the sensing reagents in the control and the test regions out to the other flow channels.

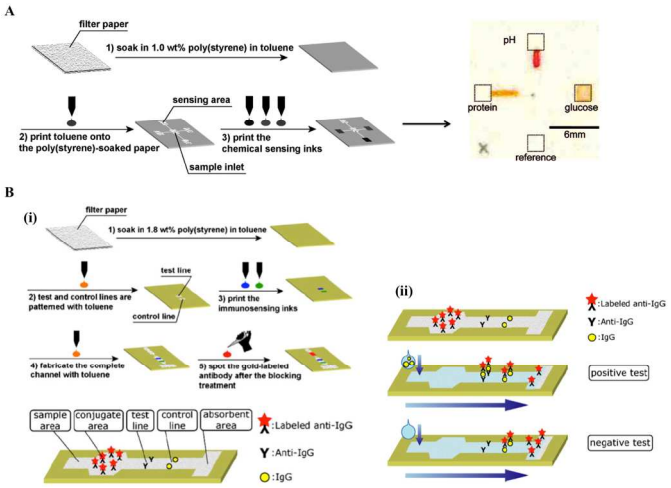

Fig. 11 Inkjet fabricated biosensors with indirect channel or zone patterning. A: schematic representation of the fabrication process of the inkjet printed multi-analyte biosensor. From Ref. [63]. Copyright 2008 American Chemical Society. B: (i) schematic representation of the fabrication process of the inkjet printed unassembled lateral flow biosensor. (ii) Reaction scheme of the sandwich assay. From Ref. [83]. Copyright 2008 Springer-Verlag.

Direct inkjet patterning as channels or zones

Since the matrix for fabricating the biosensors is usually porous and hydrophilic in nature (*e.g.* a cellulose membrane), an alternative way of patterning channels is to directly print the hydrophobic portion while leaving the hydrophilic matrix unprinted as reaction channels or zones. Using this direct approach, Li *et al.*[145] manufactured a practical and economic paper-based blood typing device that was capable of detecting all eight blood types (A+, A−, B+, B−, O+, O−, AB+, and AB−, Fig. 12A (v)). The alkenyl ketene dimer (in n-heptane) hydrophobized the unprinted hydrophilic regions as letters or symbols to represent the blood type. The detection was based on haemagglutination reactions, where a colored blood type pattern would display if there were interactions between the antigens in the blood sample and the corresponding antibodies within the letters and symbols after a saline washing step (Fig. 12A (i-v)).[145] Recently, Li *et al.*[146] also successfully showed the detection of secondary human blood groups using the same patterning approach. Even **more complex designed patterns** can also be patterned through the direct patterning approach. Figure 12B shows some of the more complicated graphic designs by Li *et al.*[147]. The patterning *via* such approach is flexible, because the hydrophobic pattern can be simply designed by drawing software (*e.g.* Microsoft PowerPoint), and then executed by an inkjet printer.

Li *et al.*[81] demonstrated that even **switches or valves** could be built into paper-based biosensors using directly patterned paper (Fig. 12C). The inkjet printed hydrophobic areas were resistant enough to confine the solution within the hydrophilic channel, yet allowed the solution to flow smoothly through the channel to the adjacent reaction zone when the switch was pressed down.

The direct patterning strategy also permits 3D paper-based biosensor development. For example, by patterning the whole reaction channels or zones into one piece of paper, a 3D biosensor was generated by folding the paper so that the corresponding channels or zones overlapped (Fig. 12D).[148, 149] The testing reagent was applied to a reaction zone, and flowed through to folded areas that overlapped with the original zone.[148, 149] In another example, the reaction channels or zones were patterned on different pieces of paper, which were then stacked together to form an overlapping reaction chamber (Fig. 12E).[150, 151] In both these 3D biosensor designs, the inkjet printer precisely deposited the reagent(s) into desired positions on the matrix. The patterned hydrophobic region not only served as a rigid structure support, but also hydrophobized to direct the flow of the hydrophilic solutions.

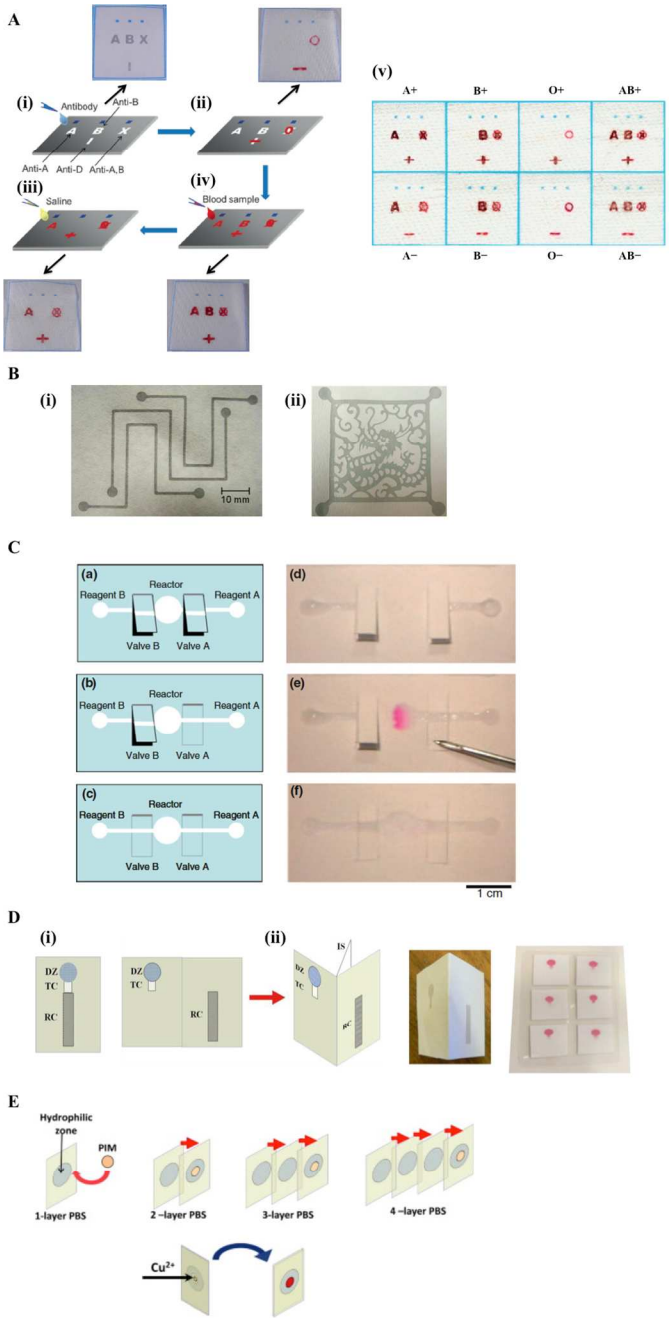

Fig. 12 Inkjet fabricated biosensors with direct channel or zone patterning. A: total inkjet fabricated blood typing biosensors. (i-v) Fabrication and testing procedures of the text-reporting blood-typing devices. (i) Anti-A and Anti-B are introduced into the corresponding letters. An equal-volume mixture of Anti-A and Anti-B is introduced into "×", and Anti-D is introduced into "I". (ii) Letter "O" and symbol "–" are printed over "×" and "–", respectively, using a non-bioactive and water-insoluble ink. (iii) A blood sample is introduced in the device for blood typing test. (iv) The blood typing result is displayed after washing with saline solution. (v) The actual tests of all eight ABO rhesus blood types. From Ref. [145]. Copyright 2012 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. B: more complicated pattern designs using the direct approach by inkjet patterning (i) multi-lined pattern and (ii) Chinese paper cut pattern of a dragon. From Ref. [147]. Copyright 2010 Elsevier B.V.. C: inkjet printed biosensor that contains switches or valves. (a–c) A design of a simple paper-based microfluidic reactor consisting of two sample dosing sites, two valves, and one central reaction site; (d–f) A paper-based microfluidic reactor based on this design was tested using acid–base neutralization reaction. (d) Phenolphthalein indicator solution was deposited onto the central reaction zone. NaOH and HCl solutions were added into reagent zones A and B, respectively. (e) NaOH solution was introduced into the reaction zone to trigger color change. (f) HCl solution was introduced later into the reaction zone *via* valve B to neutralize NaOH in the reaction zone) From Ref. [81]. Copyright 2010, Springer Science+Business Media B.V.. D: 3D biosensor by folding the inkjet patterned paper. (i) Schematic diagrams of the proposed 2D biosensor. (ii) Schematic diagrams and real images of the proposed 3D biosensors. From Ref. [149]. Copyright 2014 American Chemical Society. E: 3D biosensor by stacking the inkjet patterned paper. The paper was inkjet patterned while leaving the hydrophilic matrix unprinted. The hydrophilic zones were impregnated with PBS buffer; one of the hydrophilic zone was deposited with PIM (sensing chemical for detecting Cu^{2+}). These paper were stacked to form a 3D biosensor. From Ref. [151]. Copyright 2013 Elsevier B.V..

One problem with the above patterning approach is that the hydrophobic portion usually occupies a much larger area compared to the hydrophilic channels, resulting in excessive ink usage. To reduce the ink requirements, an alternative method is to only print a hydrophobic border to enclose the entire sensor area. Yamada *et al.* demonstrated the feasibility of this approach by printing a UV-curable ink to enclose a sampling area and a sensing area that was connected *via* a thin channel on the topside, with a fully covered backing.[85] Subsequently, they printed a top cover to conceal the hydrophilic channels (Fig. 13) and this created a functional tunnel that protected the reaction sections of the device from the environment.[84, 152] However, this fencing patterning is not as frequently used compared to printing the whole hydrophobic area, possibly because the non-fencing patterning provides a more solid barrier and rigid structure support for the biosensor, especially when the matrix of the biosensor is not rigid (*e.g.* paper-based).

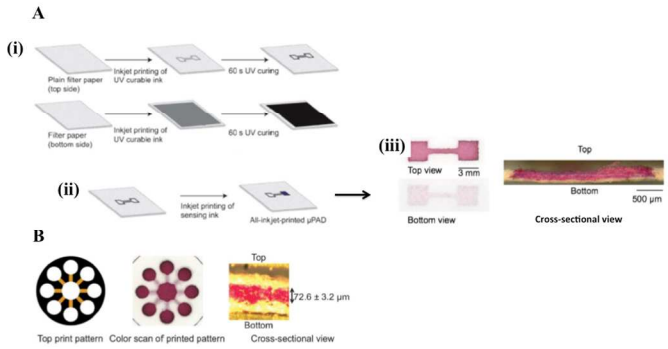

Fig. 13 Inkjet fabricated biosensors with direct channel or zone patterning by hydrophobic fencing. A: schematic representation of the fabrication process of the inkjet printed peroxide biosensor (i) patterning of the filter paper by a double-sided printing process (grey and black colors indicate the printed hydrophobic features before and after UV curing, respectively). (ii) Inkjet printing of peroxide sensing ink. (iii) Color-scanned images of the inkjet printed peroxide biosensor with a bottom cover by top, cross-sectional, and bottom views. B: inkjet printed peroxide biosensor with both a top and a bottom cover. Reproduced from Ref. [84] with permission from the Royal Society of Chemistry.

Inkjet patterning on non-porous matrices

As opposed to the patterning strategy upon porous (usually hydrophilic) surfaces, patterning upon non-porous surfaces requires different considerations. In particular, hydrophilic ink can be printed onto non-porous hydrophobic surfaces. In this case, the printed hydrophilic ink directly forms a pattern (channel or zone) on the hydrophobic matrix through adhesion (as opposed to forming a channel between hydrophobic ink and hydrophilic matrix as described above). However, poor adhesion can cause difficulties, resulting in the need to modify the surface with certain reagents, which not only improves the adhesion but also other properties (*e.g.* wettability, hydrophobicity). Wu *et al.*[153] fabricated a glucose biosensor by inkjet printing silver nanoparticles upon a PDMS modified surface (by (3-mercaptopropyl)trimethoxysilane, MPTMS). The nanoparticles showed good adhesion to the modified PDMS surface *via* a soaking test (the pattern was immersed into water for 2 h), a blowing test (the pattern was blown with an air stream of 0.5 MPa) and ultrasonication test (the pattern was placed into a water beaker and ultrasonicated). A comparison was also made to the plasma treatment patterning strategy. Although the plasma-treated PDMS allowed silver nanoparticle droplets to form more coherent patterns, the pattern failed the soaking test because of the extremely weak adhesion between the plasma-treated PDMS surface and the silver nanoparticle-based ink.[153]

Another problem for inkjet patterning upon a non-porous matrix is that the adjacent ink droplets tend to coalesce, thereby affect the quality of patterning. Optimization of the ink drop spacing can help solve this coalescence problem.[153-155] Wu *et al*. found that a drop spacing greater than 38 μm did not cause coalescence of the silver nanoparticles on the MPTMS modified PDMS surface.[153] Instead of changing the ink base (to be a reactive one, see the section on ink bases) to solve the coalescence problem, they applied a discontinuous overlapping printing strategy, which was to stagger the printing of the ink droplets. Once the first round of printing droplets dried, a second round followed to deposit the ink droplets next to the dried ones, and this continued for subsequent additions. At the end, all the ink droplets would overlap without interfering with each other to obtain a uniform pattern.[153] Belgardt *et al.* also stated that the isolated droplets (without coalescence) printed on non-porous matrix improved edge sharpness, and more continuous distribution of the effective wettability by the hydrophilic ink patterned channels was obtained.[155]

Inkjet patterning on superhydrophobic matrices

A major advantage of inkjet patterning with hydrophilic ink upon a hydrophobic non-porous matrix is that fewer assay reagents are lost in the transporting channels before they reach the detection zones.[109] This is because the liquid does not adhere to the non-porous matrix rather than absorbing into a hydrophilic porous matrix as it travels along the channels. Thus, liquid wettability is more controlled with inkjet patterned hydrophobic non-porous matrix.

An example of controlled wettability is inkjet patterning using superhydorphobic matrices. This surface has a contact angle of >150° (compared to hydrophobic matrices, where the contact angle is ~90°). On a superhydrophobic matrix, liquid tends to roll off and aggregates into droplets, analogously to the effect of water on a lotus leaf. Such phenomena are advantageous for reagent transfer, mixing or sample splitting on a biosensor. A proof-of-concept using inkjet patterning was first tested by Balu *et al.*[156], where hydrophilic black phaser ink was deposited as dot and line patterns on superhydrophobic handsheets. The water droplets (colored with food dye for visualization) showed efficient transferring, mixing, and splitting along the black phaser ink patterned channels simply by slightly tilting the patterned matrix. Stemming from Balu *et al.*'s prototype, several research groups have focused on improving the droplet mobility by controlling the droplet hysteresis level in a detection zone before it wets the next detection area.[109-111] This improvement was achieved by inkjet printing different hydrophilic ink densities in the desired detection zones respectively. The resultant biosensor allowed more focused sensing and required less reagent volumes.

Critical parameters for inkjet patterning and post-inkjet fabrication of biosensors

As seen in the above sections, there is great depth and variety to patterning that can be achieved using inkjet printing. When developing for new applications, there are some key parameters to consider both for patterning and also for post-testing once the ink-jet fabrication of biosensors is completed.

For most chemists, the choice of surface (*e.g.* hydrophilic, hydrophobic, porous and non-porous) is paramount, and highly dependent on the final application. However, this surface also critically determines the type of patterning strategy, which may require pre-treatment or modification of the surface to permit a better printing quality or to adopt a certain bio-activity for the final biosensor. Thus bonding of the ink to the surface becomes the next consideration (see the section on ink properties), which also drives the choice of printer (as described in the section on printer choices). The complex interactions between the matrix, the ink, and the printer allow achievement of the required resolution.

After considering the surface, ink, and printer interplay, the next step is to choose a suitable reagent for patterning the channels or zones. On a porous matrix, the choice of hydrophobizing reagent for patterning relates to its mechanism of grafting, and is divided into three categories: (1) physical blocking of the pores in paper (*e.g.* using photoresist or PDMS); (2) physical deposition of a hydrophobizing agent (*e.g.* polystyrene or wax); and (3) chemical

modification of porous matrix (*e.g.* Alkyl ketene dimers = AKD and alkenyl succinic anhydride = ASA, both are cellulose reactive agents).[157] The hydrophobization through the chemical modification is more stable than that of the physical ones, because the modification cannot be removed by organic solvent extraction.[158] For example, wax does not tolerate strong acids or bases, and is not compatible with organic solvents.[130, 132] Therefore, a reagent tolerability test for the inkjet patterned barriers should be conducted before performing the subsequent sensing test. While wax and AKD are the two commonly used hydrophobizing reagents for inkjet patterning, other newly developed hydrophobizing reagents have displayed superior performances. These include methylsilsesquioxane (MSQ)[159], silicon resin[160], and Teflon[161]. All these new hydrophobizing reagents were tolerant to organic solvents (*e.g.* glycerol, toluene, piperidine, Trifluoroacetic acid = TFA), lower surface energy solvents (*e.g.* Dimethyl sulfoxide = DMSO), and surfactant solutions (*e.g.* SDS, Triton X-100, CTAB = hexadecyltrimethylammonium bromide).[159-161]

Patterning on non-porous surfaces hinges on the adhesion of the ink to the matrix and droplet coalescence issues. The adhesion can be strengthened by including additives in the ink formulation and /or modifying the matrix with suitable reagent(s).[153] Droplet coalescence can be solved *via* optimization of the minimum drop spacing, or adopting a staggered printing manner (mentioned in inkjet patterning on non-porous matrices section). It is noteworthy that sintering maybe necessary after inkjet patterning. This is especially true for conductive inks, as they require sintering (a thermal treatment) to form the connections between neighbouring molecules, which improves electrical conductivity and mechanical adhesion. Some hydrophobizing reagents (*e.g.* AKD and silicon resin) also showed better performance after sintering.[160, 162]

Finally, other parameters that improve inkjet patterning include optimizing the number of printing cycles to allow maximum ink coverage; double-sided printing (top and bottom) to allow full hydrophobization on porous matrix *via* ink penetration and coalescence;[163] and optimizing reagent printing order during multiple layered printing to preserve optimal activity of the biomolecules.[164, 165]

Highlights of functionality features by inkjet printing

Beyond the added convenience of biosensor fabrication via inkjet printing, the technology is providing a transformative platform to reach new frontiers in biosensor manufacturing. While described in detail via a manufacturing perspective in the preceding sections, some of the most significant advances in regards to new functionality achieved by inkjet printing of biosensors include:

- **Switches and valves**[81] (Fig. 12C). Inkjet printed hydrophobic barriers are flexible and resistant enough to regulate liquid flow between different reaction zones. This is a good imitation of the sophisticated "Lab-on-chip" (or microfluidics) system but with a lower cost and simpler fabrication.

- **Three-dimensional biosensor *via* folding or stacking**[148-151] (Fig. 12D and 12E). Inkjet-patterned shapes can be overlapped to guide the directions of liquid flow into different detection zones. The resulting folded or stacked 3D biosensors adopt a flow-through detection; this eases the liquid movement *via* gravitational forces and minimizes the device size.

- **Controlled sequential mixing**[108] (Fig. 14A). Inkjet printed neighboring reagents can be deposited to not interfere with each other when stored on the device in dried form. Mixing of these reagents then occurs when liquid is added and moves along the printed regions. This alleviates the requirements for pre-mixing reagents prior to assay commencement.

- **Erasable enzyme-enabled 2D code**[166] (Fig. 14B). Gradient printing by varying the ink deposition density allows development of an enzyme 2D code. This code appears after shortly after initiation of the reaction, formed by different enzyme reaction rates between "black" and "white" areas (which represent inkjet printing of two different gradients of enzyme and substrate mixture at a different ratios). The signal disappears when the enzyme substrate is depleted.

- **Microchannel built within a liquid matrix**[167] (Fig. 14C). Ink deposited with a lower density than the liquid matrix can coalesce to form desired shapes as microchannels. This transforms microfluidics fabrication by reducing cost and simplifying fabrication.

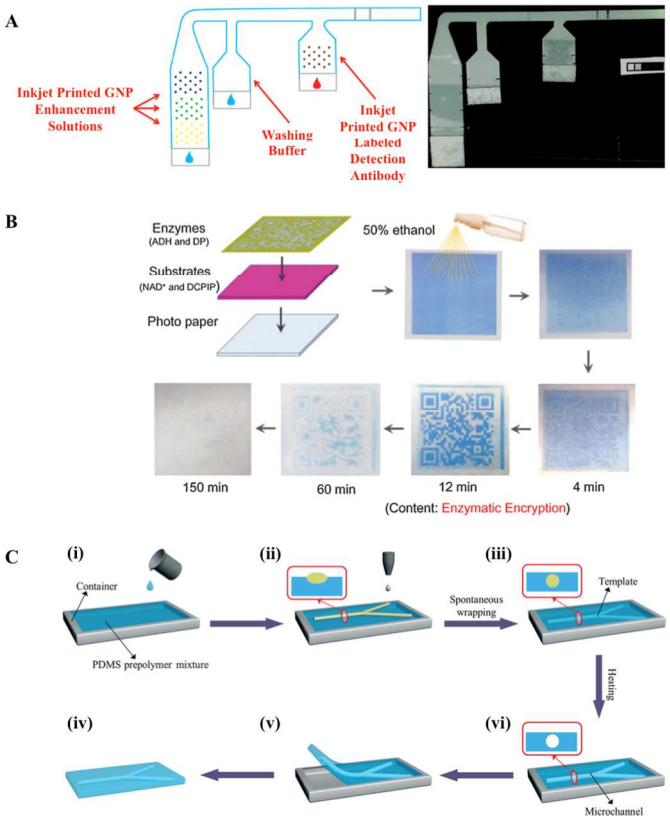

Fig. 14 Some functional advances in biosensors that were realised by inkjet printing. A: Sequential mixing of signal enhancement solutions in a 2D paper network biosensor. Three signal enhancement solutions were inkjet printed next to each other. Mixing only occurs when solution is applied, and in turns, wicks along the printed regions. From Ref. [108]. Copyright 2014 American Chemical Society. B: An erasable enzyme-enabled 2D code. The coding was achieved by inkjet printing two different gradients of enzyme and substrate mixture (at a different ratios). The decoding was initiated by spraying the paper with a water/solvent mixture

(50% ethanol). The enzyme 2D code appeared at maximum intensity after 12 minutes, and disappeared after 150 minutes when the printed enzyme substrate was depleted. From Ref. [166]. Copyright 2014 Royal Society of Chemistry. C: Inkjet fabrication of a microfluidic reactor on a liquid template involved the following steps: (i) Pouring a PDMS prepolymer mixture into a container; (ii) printing a Y-shape pattern on the surface of the prepolymer mixture (inset: relative position sketch of the pattern and prepolymer mixture surface); (iii) standing for a few seconds to allow the pattern to be wrapped spontaneously; (iv) after heating the prepolymer mixture with the liquid template, the prepolymer mixture thermally cures and the liquid template evaporates, leaving the microchannel in the PDMS matrix; (v) peeling off the fabricated PDMS matrix containing the microchannel; (vi) the typical microfluidic reactor in the PDMS matrix. From Ref. [167]. Copyright 2015 Royal Society of Chemistry.

Conclusions

Due to the simple, rapid, flexible, high resolution, low cost, and efficient properties for mass production, inkjet printing is becoming a routine tool for biosensor fabrication as this printing technology matures.[19] It has been nearly 30 years since the first report of an inkjet printed biosensor.[168] Since then the diversity in terms of ink, matrix, and patterning methods has dramatically increased. Inkjet printing is now a functional printing method, and a competitive fabrication tool for biosensor manufacturing, especially for point-of-care diagnostic biosensors. The variety of inkjet printers available enable both fabrication of smart biosensors (*e.g.* electronics) that require high-resolution deposition (*e.g.* EHD inkjet printer), and fabrication of common biosensors (*e.g.* glucose biosensor) in an efficient way and at a low cost. Numerous partially inkjet printed biosensors have been reported, however, fully inkjet printed biosensors have also been realized, which enables rapid mass production because the whole fabrication process is realized within a single machine.

The potential of inkjet printing in this burgeoning field, however, is still largely untapped, and we anticipate that even more possibilities and novelties are highly likely to emerge. For instance, developing automated processes to test and adjust inks (such as rheological measurements) would greatly aid the inkjet printing process to develop a step further and increase the efficiency of biosensor fabrication. Miniaturization of the inkjet printer to be a portable machine is another issue, so that the printing process can even be conducted in field. Perhaps even more critical, however, is to integrate inkjet printing with other analytical processes for advanced and fully-automated analytical systems. The potential of inkjet printing should not be underestimated – the technology is being applied to a variety of fields, and its development is becoming more versatile as multi-disciplinary knowledge collides.

Acknowledgements

We thank John Bartlett and David McMillan for support and advice, and Richard Burns for careful reading of this manuscript. This work was funded by the Queensland Government, Department of Science, Information Technology, Innovation and the Arts (DSITIA, Australia).

Notes

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Table 2 Examples of fully or partially inkjet printed biosensors, and the details of their fabrication

Fully or partial inkjet printed biosensor(s)	Inkjet patterning strategy	Inkjet printer(s)	Ink for Patterning	Ink for Sensing	Types of inkjet printed biosensor(s)	References
Partial (for patterning)	Multi-layers surface inkjet patterning	Thermal Canon inkjet printer (i905D)	NA	Biological ink (dissolving 1.7 mg/ml of HRP in a 0.1 M phosphate buffer, pH 6.5, which contained EDTA 1.5 mM as antimicrobial agent and 10% (w/v) of glycerol as stabilizer; electronic ink (diluting 20 ml of the 1.3 wt.% PEDOT/PSS dispersion in distilled water, to a final volume of 50 ml)	Hydrogen peroxide biosensor	112
Partial (for patterning)	Multi-layers surface inkjet patterning	Thermal Canon inkjet printer (i905D)	NA	Biological ink (dissolving 0.6 or 6 mg/ml of GOD in a 0.1 M phosphate buffer, pH 6.5, which contained EDTA 1.5 mM as antimicrobial agent and 10% (w/v) of glycerol as stabilizer); electronic ink (diluting 20 ml of the 1.3 wt.% PEDOT/PSS dispersion in distilled water, to a final volume of 50 ml	Glucose biosensor	58
Partial (for reagents dispensing)	NA	Piezoelectric inkjet (Dimatix)	NA	20 wt% CuO nanoparticles (5-8 nm) in a mixed solvent of deionized water, ethanol, isopropyl alcohol, and ethylene glycol in the ratio of 50:20:5:5 vol%	Glucose sensor	80
Fully	Multi-layers surface (entrapment) inkjet patterning	Piezoelectric Dimatix Materials Printer 2800 (DMP 2800)	A polypyrrole (PPy) bottom layer; ethyl cellulose (EC, 0.5% w/v in butanol solution); A nonconductive dielectric layer (Electrodag 452 SS BLUE) worked as insulation layer	PPy/HRP (2.5 mg of HRP dissolved in 1 ml of PPy dispersion); PPy/GOD (5 mg of GOD dissolved in 1 ml of PPy)	Glucose biosensor	115
Partial (for patterning)	Direct inkjet patterning of hydrophilic ink upon hydrophobic surface	Piezoelectric Dimatix inkjet printer (Fujifilm 2831 series)	Silver nanoparticles	NA	Glucose sensor	153
Fully	Indirect reaction channel or zone inkjet patterning	(Piezoelectric) PicoJet-2000 device from Microjet (Shiojiri, Nagano, Japan)	Toluene (to dissolve the polystyrene treated filter paper)	0.2 mg/ml human IgG in water (control line); 1.22 mg/ml anti-human IgG in water (test line)	Lateral flow biosensor	83
Fully	Multi-layers surface (entrapment) inkjet patterning	Piezoelectric inkjet printer (Model DMP-2800, Fujifilm Dimatix, Inc, Japan)	Polyvinylamine (PVAm) underlayer directly onto the paper surface; silica sol intermediate layer; silica sol overlayer	a buffered enzyme solution that contained acetylcholineserase (AChE, 50 U/ml) and 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) (500 μM)	Lateral flow dipstick	117
Fully	Multi-layers surface (entrapment) inkjet patterning	Piezoelectric inkjet printer (Model DMP-2800, Fujifilm Dimatix, Inc, Japan)	PVAm layer, intermedium and overlayer of sol-gel silica	Acetylcholineserase (AChE) (in the sensing region) and indophenyl acetate (IPA) (in the substrate region) sandwiched between the two layers of sol-gel silica	Lateral flow dipstick	118
Fully	Multi-layers surface (entrapment) inkjet patterning	Piezoelectric inkjet printer (Model DMP-2800, Fujifilm Dimatix, Inc, Japan)	Sol-gel silica to entrap enzymes in the substrate and sensing zones. Methyltrimethoxysilane (MTMS) for hydrophobic barrier (HB zone)	Chlorophenol red β-galactopyranoside (CPRG) and β-galactosidase (β-GAL) in the substrate and sensing zones, respectively	Bi-directional lateral flow dipstick	114

NA: not applicable

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Table 2 Summary of some fully or partial inkjet printed biosensors (continued)

Fully or partial inkjet printed biosensor(s)	Inkjet patterning strategy	Inkjet printer(s)	Ink for Patterning	Ink for Sensing	Types of inkjet printed biosensor(s)	References
Fully	Direct non-fencing reaction channel or zone inkjet patterning	Piezoelectric EPSON Workforce 30 inkjet printer	10% hexadecenyl succinic anhydride (ASA) in hexanol	Silver nanoparticles (with glycerol in a volume ratio of 2:5 glycerol/colloid solutiion)	Surface enhanced raman spectroscopy (SERS) lateral flow sensor	169
Partial (for reagents dispensing)	NA	Piezoelectric EPSON Workforce 30 inkjet printer	NA	Gold nanoparticles (GNP, glycerol and ethanol in a volume ratio of 5:4:1)	SERS lateral flow sensor	170
Partial (for reagents dispensing)	NA	Piezoelectric EPSON Workforce 30 inkjet printer	NA	Silver nanoparticles (with 40% glycerol and 10% ethanol)	SERS lateral flow sensor	171
Partial (for reagents dispensing)	NA	Piezoelectric inkjet printer (Scienion, Berlin, Germany)	NA	A murine antibody to Plasmodium falciparum histidine rich protein 2 (PfHRP2) at 1 mg/ml at the test line; an anti-mouse antibody at 0.1 mg/ml at the control line	2D paper network lateral flow biosensor	172
Partial (for reagents dispensing)	NA	Piezoelectri spotter (SciFLEXARRAYER S3, Scienion AG)	NA	Mouse monoclonal anti-PfHRP2 IgM (0.375 µl, 1 mg/ml) at the test line; ImmunoPure Antitoby goat anti-mouse IgG (0.375 µl, 0.5 mg/ml) at the control line; 2 µl of each "enhancer" solution, "activator" solution, and "initiator" solution on the third inlet	2D paper network lateral flow biosensor	108
Partial (for patterning)	Direct non-fencing reaction channel or zone inkjet patterning	Thermal CanonTMiP4700 inkjet printer	4% Alkenyl ketene dimer (AKD)	NA	2D and 3D sensors by stacking	151
Partial (for patterning)	Direct non-fencing reaction channel or zone inkjet patterning	Thermal CanonTMiP4700 inkjet printer	4% AKD in n-heptane	NA	2D and 3D sensors by stacking	149
Partial (for patterning)	Direct non-fencing reaction channel or zone inkjet patterning	Thermal CanonTMiP4700 inkjet printer	4% AKD in n-heptane	NA	2D and 3D sensors by folding	148
Partial (for patterning)	Direct non-fencing reaction channel or zone inkjet patterning	Thermal CanonTMiP4700 inkjet printer	4% AKD in n-heptane	NA	2D and 3D sensors by folding	150
Fully	Direct fencing reaction channel or zone inkjet patterning	Piezoelectric EPSON PX-101 inkjet printer (Seiko Epson, Suwa, Japan)	Non-volatile UV-curable ink (59.5% octadecyl acrylate, 25.5% 1,10-Decanediol diacrylate, and 15% Irgacure 651)	H_2O_2 sensing ink (1.0 ml of 2.8 mg/l HRP in citrate-phosphate buffer (pH 7.0), 2.0 ml of 7.5 mM 3,3',5,5'-Tetramethylbenzidine (TMB) in 2-propanol, and 1.0 g of glycerin to control the viscosity)	Single-analyte chemical sensor	84
Fully	Direct fencing reaction channel or zone inkjet patterning	Piezoelectric EPSON PX-105 inkjet printer (Epson, Suwa, Japan) for patterning microfluidic structures; Piezoelectric Dimatix DMP 2831 (Dimatix Fujifilm Inc., Santa Clara, USA) for depositing reagents	Octadecyl acrylate and 1,10-decanediol diacrylate UV-curable ink	1 mM $TbCl_3$ solution with 15 vol% ethylene glycol (sensing areas); 25 mM $NaHCO_3$ (sampling areas)	Single-analyte chemical sensor	85

Table 2 Summary of some fully or partial inkjet printed biosensors (continued)

Fully or partial inkjet printed biosensor(s)	Inkjet patterning strategy	Inkjet printer(s)	Ink for Patterning	Ink for Sensing	Types of inkjet printed biosensor(s)	References
Fully	Indirect reaction channel or zone inkjet patterning	(Piezoelectric) PicoJet-2000 device from Microjet (Shiojiri, Nagano, Japan)	Toluene (to dissolve the polystyrene treated filter paper)	pH-responsive ink; protein-sensitive ink; glucose-sensitive ink	Multi-analyte chemical biosensor	63
Fully	Direct non-fencing reaction channel or zone inkjet patterning	Thermal Canon inkjet printer (Pixma ip4500)	AKD in 2% (w/v) heptane	Anti-A, clone 10090; Anti-B, clone 10091; and Anti-D, clone 20093 were introduced into the hydrophilic patterns "A", "B", and "/"	Blood typing biosensor	145
Partial (for patterning)	Direct non-fencing reaction channel or zone inkjet patterning	Thermal Canon inkjet printer (Pixma ip4500)	AKD in n-heptane	NA	Blood typing biosensor	146
Fully	Direct non-fencing reaction channel or zone inkjet patterning	Thermal Canon inkjet printer (Pixma ip4500)	AKD in heptane	No^{2-} as indicator and alkaline phosphatase	Microfluidic analytical sensor	147
Partial (for patterning)	Direct non-fencing reaction channel or zone inkjet patterning	Thermal Canon inkjet printer (Pixma ip4500)	AKD in n-heptane	NA	Microfluidic analytical sensor	81
Partial (for patterning)	Direct non-fencing reaction channel or zone inkjet patterning	Thermal Canon inkjet printer (Pixma ip4500)	AKD in 2% (w/v) heptane	NA	Microfluidic analytical sensor	82
Partial (for reagent dispensing)	NA	Piezoelectric DMP-2831 material printer (Fujifilm Dimatix, Santa Clara, CA)	NA	Two types of dye-encapsulating polymer nanoparticle emulsions (pBzMA or p(DEGMMA-co-MMA)) in mixing ratio (100:0, 80:20, 60:40, 40:60, 20:80, and 0:100); The final ink composition corresponds to a polymer solid content of 10 mg/ml in H_2O with 10% (v/v) of ethylene glycol	Strip paper sensor	173
Partial (for reagents dispensing)	NA	Thermal Canon inkjet printer (Pixma ip4500)	NA	Albumin-FITC (FITC = fluorescein isothiocyanate) in buffer with 1.0 mg/ml HRP	Paper biosensor	174
Partial (for reagents dispensing)	NA	Thermal inkjet office printer (HP Deskjet D2360)	NA	4BCMU emulsion ink (4 wt% of 4BCMU, 23 wt% of 1,2,4-trimethylbenzene, 10 wt% of SDS, 37 wt% of 1-propanol and 26 wt% of water)	Paper sensor	175
Partial (for reagents dispensing)	NA	Thermal inkjet office printer (HP Deskjet D2360)	NA	Diacetylene, bisurea and olitoethylene oxide	Paper sensor	176
Partial (for reagents dispensing)	NA	(Piezoelectric) MicroFab JetLab4 system (MicroFab Technologies Inc.)	NA	TiO_2 nanoparticels and Ag nanoparticles	Printed-paper-based memory devices	177

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