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## A review of digital microfluidics as portable platforms for lab-on-a-chip applications

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Following the development of microfluidic systems, there has been a high tendency towards developing lab-on-a-chip devices for biochemical applications. A great deal of effort has been devoted to improve and advance these devices with the goal of performing complete sets of biochemical assays on the device and possibly developing portable platforms for point of care applications. Among the different microfluidic systems used for such a purpose, digital microfluidics (DMF) shows high flexibility and capability of performing multiplex and parallel biochemical operations, and hence, has been considered as a suitable candidate for lab-on-a-chip applications. In this review, we discuss the most recent advances in the DMF platforms, and evaluate the feasibility of developing multifunctional packages for performing complete sets of processes of biochemical assays, particularly for point-of-care applications. The progress in the development of DMF systems is reviewed from eight different aspects, including device fabrication, basic fluidic operations, automation, manipulation of biological samples, advanced operations, detection, biological applications, and finally, packaging and portability of the DMF devices. Success in developing the lab-on-a-chip DMF devices will be concluded based on the advances achieved in each of these aspects.

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### 1. Introduction

In recent years, a prodigious number of studies has been devoted to advance microfluidic devices that can replace the

conventional laboratory processes performed by technicians and experts in large scale laboratories.<sup>1</sup> The primary advantages of such devices are automation and reduction of the consumed samples and reagents. In addition, the large surface-to-volume ratio (reducing the reaction time) and high controllability in sample manipulation are the other features of such devices.<sup>1</sup> These advantages have made microfluidic systems suitable for performing biochemical experiments. Following the first attempt in developing a microfluidic

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system in the 1950s,<sup>2</sup> several mechanisms have been used to drive and control the flow in microfluidic systems.<sup>1</sup> According to Haeberle and Zengerle,<sup>1</sup> the seminal microfluidic systems that have been developed for lab-on-a-chip applications are categorized as: capillary driven test strips,<sup>3</sup> pressure driven systems,<sup>4</sup> centrifugal microfluidic devices,<sup>5</sup> electrokinetic platforms,<sup>6</sup> droplet-based (continuous and digital) microfluidic systems,<sup>7–9</sup> and non-contact dispensing systems.<sup>10</sup> Despite the common features of these systems, the mechanism of fluid manipulation and the application range of each system are different, and consequently each system has advantages and shortcomings compared to other systems. The review of the aforementioned systems shows that most of these devices have 3D geometries (including pre-etched or machined microchannels/flow passages) and integrated external modules (e.g., syringe pumps and electrical actuators)<sup>3–10</sup> that make the fabrication of the device complicated. Also, in most cases the platform is limited to a certain application, and the device cannot be used for multiple purposes. In addition, the miniaturization of all the assay-processing steps on a single device, required for developing a portable point-of-care device, is difficult. On the other hand, digital microfluidics (DMF),<sup>9,11–13</sup> which is a droplet-based microfluidic system with a planar geometry can be simply fabricated by photolithography.<sup>11,12</sup> The fluid manipulation mechanism on these systems (in the form of actuation of discrete droplets) does not require external modules or complicated geometries such as pumps or valves.<sup>9,14</sup> Another unique feature of DMF is that the actuating electrodes on the chip can be fabricated on a 2D array with a general electrode design, and as a result, a single platform can be reconfigured for multiple applications.<sup>11,12,15</sup> The droplet-based characteristics of DMF allows for parallel operations on a platform,<sup>16,17</sup> increasing the speed of assay-processing steps.<sup>17</sup> Furthermore, a signifi-

cantly high precision can be achieved for sample preparation on such systems.<sup>18–20</sup> Therefore, DMF has the potential to be further developed for mass production and be merged as a device of choice for point-of-care applications. It has to be emphasized that each of the microfluidic systems mentioned above are advantageous for certain applications, and the purpose of this review is not to suggest DMF as a replacement for other systems. This review rather intends to evaluate DMF as a reliable system for future development and commercial production for a wide range of applications.

In digital microfluidic devices, which are categorized into open and closed configurations,<sup>21–24</sup> droplets can be manipulated using several different techniques such as electro-wetting on dielectric (EWOD),<sup>9,25</sup> dielectrophoresis (DEP),<sup>26,27</sup> surface acoustic waves,<sup>28,29</sup> magnetic force,<sup>30,31</sup> thermocapillary force,<sup>32,33</sup> optoelectrowetting<sup>34,35</sup> and magnetic actuation of liquid marbles.<sup>36</sup> Despite the general success of all of these methods, EWOD offers the most flexible and best functionality for droplet actuation, and has been extensively used for lab-on-a-chip applications.<sup>11,12</sup>

In EWOD-based DMF devices, the electrodes are patterned on a substrate (normally a glass slide or a silicon wafer with thermally grown oxide layer) using photolithography and then covered with a dielectric and a hydrophobic layer.<sup>9</sup> In the closed configuration, the droplet is sandwiched by a top plate which is usually an ITO-coated glass slide used as a transparent electrode, covered with a hydrophobic layer. Droplet manipulation is performed by applying a DC or AC voltage to the system.<sup>9,14</sup> A detailed review of EWOD can be found in ref. 37 and the references therein.

After the introduction of the primary DMF platforms,<sup>9,38,39</sup> a large number of studies have been performed on enhancing and advancing these devices for lab-on-a-chip applications. The common purpose of these studies have been to develop a package capable of performing a complete set of operations required for biological assays, and in some cases, develop a portable device for point-of-care applications. Multiple reviews have reported earlier advances for general,<sup>11,40,41</sup> or specific applications of DMF in areas related to electrochemistry,<sup>42</sup> clinical diagnostics,<sup>43</sup> cell-based applications<sup>44,45</sup> and detection or sensing.<sup>46</sup> In this review paper, the most recent and major advances on all aspects of DMF and its applications in lab-on-a-chip devices are presented. Also, this review provides a “critical” evaluation of the potential of DMF-based lab-on-a-chip devices as multifunctional tools for performing multiple biological assays as well as the possibility of the application of these devices for point-of-care applications. For this purpose, the advances in DMF-based devices are categorized into eight major streams including device fabrication, basic fluidic operations, automation, manipulation of biological samples, advanced operations, detection, biological applications and packaging and portability. Each of these streams is thoroughly evaluated to assess the future of the DMF-based devices and their significance in point-of-care applications.



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## 2. Device fabrication

Various substrates have been used for the fabrication of DMF devices. Glass and silicon wafers with an oxidized layer (thermally grown on the surface) are commonly used as the substrate.<sup>47,48</sup> For the studies including packaging, the DMF chips have been fabricated on printed circuit boards (PCB).<sup>15,49</sup> While such substrates are beneficial for non-disposable chips, in recent years there has been a high demand for developing DMF devices with disposable substrates. Paper-based substrates are the most common disposable substrates for chip fabrication.<sup>50–54</sup> Although the quality and resolution of the fabricated electrodes are hindered on these substrates, their low cost and ease of fabrication will allow for replacement of the chip after each round of experiment, and consequently, the chance of cross contamination will significantly decrease. Most studies use screen printing for fabrication of electrodes on the paper-based substrates. Inject printing has also been used for this purpose to enhance the resolution of the fabricated electrodes as well as automating the fabrication process.<sup>53</sup> The applications of these disposable devices were also shown for Immunoassays.<sup>54</sup> Similarly, disposable DMF chips have been fabricated using inexpensive screen printing method on other flexible substrates such as polyimide foils.<sup>55</sup> Other than such flexible substrates, disposable DMF chips have been fabricated using printed circuit boards (PCB).<sup>56,57</sup> Sista *et al.*<sup>56</sup> used the PCB fabricated device for diagnosing multiple newborn diseases.

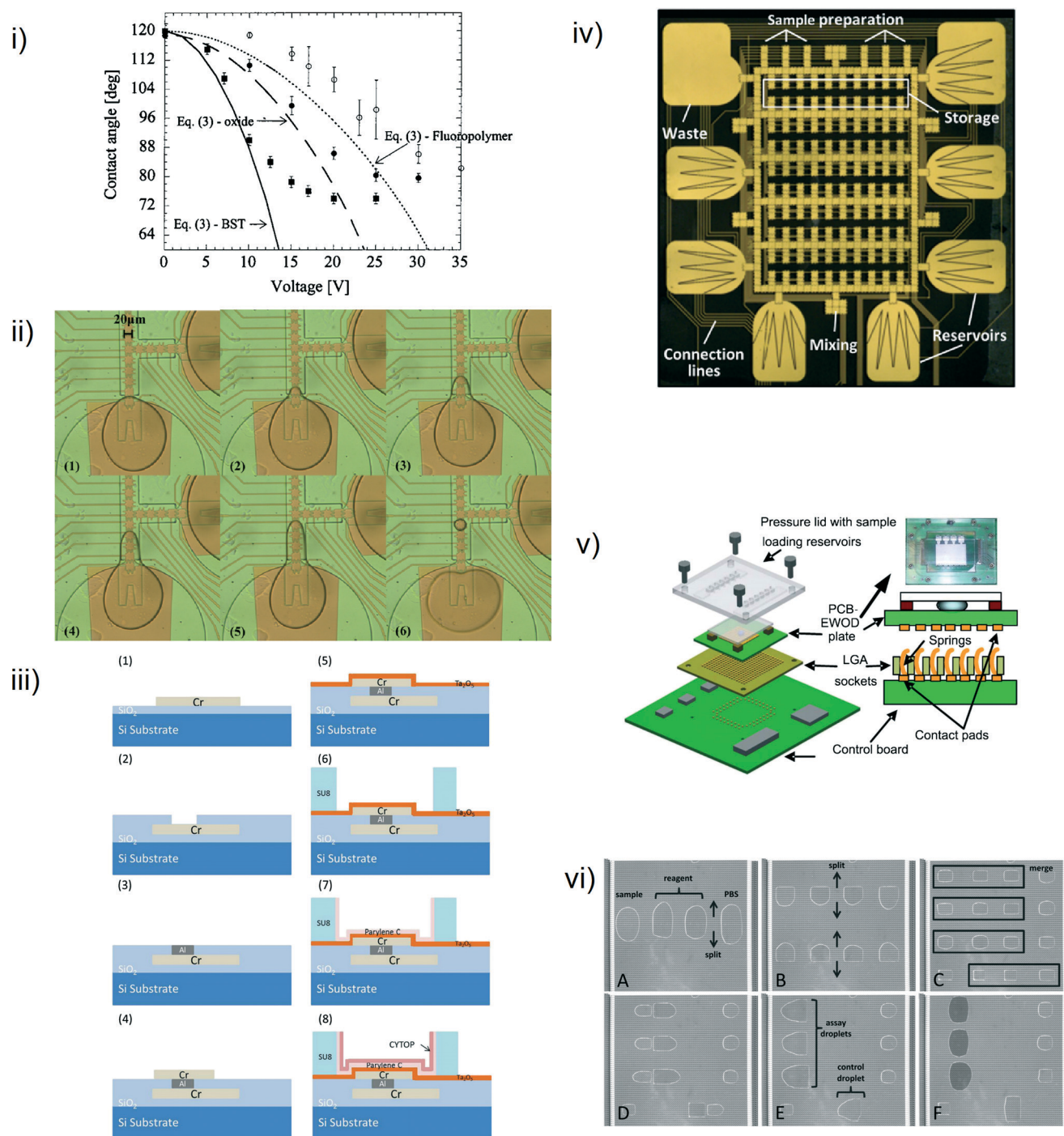
Gold is the widely used material for the electrodes. However, as the adhesion of gold to the substrate is not sufficient, a layer of chromium or titanium is usually deposited as the base layer to increase the adhesion of gold to the substrate.<sup>58</sup> Other materials such as ITO,<sup>59</sup> chromium,<sup>48</sup> silver, carbon<sup>52</sup> and copper<sup>18</sup> have also been used for patterning the electrodes. While the resolution of the electrode geometry might vary for different materials used for fabrication, the choice of the electrode material does not significantly alter the durability of the device.

Unlike the electrodes, the choice of material and the quality of deposition for the dielectric layer is very important. The required applied voltage for droplet transport and splitting is significantly dependent on the dielectric constant of the material, *i.e.* the higher the dielectric constant the lower the required voltage.<sup>37</sup> Further, the uniformity and fineness of the layer is crucial in preventing the dielectric breakdown in applying high voltages or for long actuation periods. The primary studies used the materials such as polytetrafluoroethylene (PTFE),<sup>60</sup> Parylene C<sup>61</sup> and SiO<sub>2</sub>.<sup>39</sup> Parylene C and SiO<sub>2</sub> are the most common dielectric materials due to their stability and uniformity.<sup>14,62</sup> Their dielectric constant is in a low range of 2–4 and their reported thicknesses range between 0.1–15 μm. The minimum required voltage for droplet actuation is reported as 60–80 V for 800 nm of parylene C and ~25 V for a 200 nm of SiO<sub>2</sub> layer.<sup>9,25,62</sup> However, this is the threshold voltage for droplet motion, while splitting requires higher voltages to overcome the sur-

face tension effect.<sup>14,18</sup> SU-8 and S1813 photoresists have also been used in some studies for their ease of deposition (spin coating). However, they have a low dielectric constant which ranges around 3.<sup>38,63,64</sup> Materials with a very high dielectric constant have recently been used as the dielectric layer for low-voltage droplet transport. Their high dielectric constants prevent dielectric breakdown by requiring low voltage for droplet splitting/dispensing. For instance, droplet actuation with a voltage as low as 15 V has been performed with (Ba<sub>0.7</sub>Sr<sub>0.3</sub>)TiO<sub>3</sub> (BST)<sup>65</sup> (with dielectric constant of ~180) and Ta<sub>2</sub>O<sub>5</sub> (with a dielectric constant of 25–40)<sup>66,67</sup> when they are used as the insulating layer. Fig. 1i shows the contact angle change *versus* the applied voltage for (Ba<sub>0.7</sub>Sr<sub>0.3</sub>)TiO<sub>3</sub> compared to two other insulating materials *i.e.*, silicon dioxide and a Fluoropolymer. Such materials with extremely high dielectric constants can be used for EWOD applications requiring a low actuation voltage. However, the necessity of having the hydrophobic layer on the chip as the final layer increases inevitably the required applied voltage.<sup>48</sup> These hydrophobic layers are known to have a low dielectric constant, and hence, the majority of the voltage drop occurs across this layer. Consequently, the minimum actuation voltage and breakdown of the device is limited to the hydrophobic layer coated on the insulating layer.<sup>48</sup> For instance, the minimum required voltage for BST (coated with a hydrophobic layer) is limited to 15 V.<sup>65,66</sup> Multilayer insulating layers were shown to function more effectively, and the devices are more durable with a much longer time-dependent dielectric breakdown.<sup>48,68</sup> For instance, an insulating layer was formed using a layer of high dielectric materials such as Ta<sub>2</sub>O<sub>5</sub> followed by a Parylene C and a hydrophobic layer (Fig. 1ii and iii). Thermal treatment of the Ta<sub>2</sub>O<sub>5</sub> layer significantly increased the electrical properties and the durability of the device.<sup>48</sup> Most recently, Narasimhan and Park<sup>69</sup> introduced an ion gel material with a high dielectric constant as the insulating layer which can be easily spin coated on the chip surface. The ion gel consists of poly(vinylidene fluoride-cohexafluoropropylene) [P(VDF-HFP)] as the copolymer, and 1-ethyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide [EMIM][TFSI] as the ionic liquid.

The last layer in the fabrication of the DMF devices is the hydrophobic layer commonly made of fluoropolymer-based materials such as Teflon<sup>14</sup> and CYTOP<sup>20,48,68</sup> allowing for droplet transport on the chip.<sup>9,14</sup> The contact angle reported for water on these fluoropolymers is between 105–117°. However, when used with ambient oil or silicone oil, a contact angle of up to 180° has also been reported.<sup>20</sup> The layer formed by such fluoropolymer is stable enough in aqueous solutions.<sup>70</sup> However, their hydrophobicity is compromised as they become in contact with biological samples such as proteins.<sup>70</sup> This issue will be discussed in detail in section 5.

In more recent studies, fabrication of the DMF devices with multilayer electrode-connection system has allowed for patterning a 2D array of electrodes without any limitations for the electrical access to the electrodes. In these devices,



**Fig. 1** i) Contact angle vs. applied potential for 700 Å BST (solid squares), 1000 Å silicon dioxide (solid circles) and 1200 Å fluoropolymer (open circles). All cases have a hydrophobic coating on top (reproduced from ref. 65 with permission from The American Institute of Physics). ii) A high-resolution fabricated chip with 20 μm electrodes and 1 μm thick connection lines (reproduced from ref. 68 with permission from Elsevier). iii) Schematics of the fabrication steps for a chip with multilayer insulating layer (reproduced from ref. 68 with permission from Elsevier). iv) Multilayer electrode fabrication with the connection in the bottom layer and the electrodes on the top layer (reproduced from ref. 64 with permission from The Royal Society of Chemistry). v) Use of printed circuit board (PCB) for multilayer chip fabrication (reproduced from ref. 71 with permission from IEEE). vi) 2D array of electrodes fabricated with a thin film transistor (reproduced from ref. 72 with permission from The Royal Society of Chemistry).

the connection lines are initially patterned on the substrate. An intermediate dielectric layer is then deposited with patterned holes for accessing the connection lines. On the top of

the dielectric layer, the electrodes are patterned which are connected to the connection lines through the holes in the dielectric layer.<sup>48,64,68</sup> A sample chip with multilayer electrode

fabrication is shown in Fig. 1iv. Printed circuit boards have also been used for multilayer chip fabrication (Fig. 1v),<sup>71</sup> where the electrodes can be patterned on the entire chip, enhancing multiple parallel droplet manipulation. It is also shown that with the use of a thin film transistor array for electrode fabrication, one can make a large number of electrodes in a 2D array with the capability of capacitive droplet sensing for the control of the position and the size of the droplet (Fig. 1vi).<sup>72</sup>

Recent advances in opto-electrowetting has resulted in emerging this technology for manipulation of droplets on a light-actuated electrodeless platform.<sup>73–77</sup> While the DMF platforms with such light actuated system are not as popular as EWOD-based devices, a great deal of effort has recently been devoted to increase controllability in droplet manipulation for promoting this technology for biochemical applications. This technology has benefitted from the development of optically-sensitive polymers<sup>75</sup> which potentially can also be used for 3D configurations as well as wearable device applications.

Fabrication of DMF devices in a 3D format has been illustrated in several recent studies<sup>78–82</sup> implemented different configurations of surface geometries of the DMF chip and showed improvements in LOC applications using 3D biochips in comparison with conventional 2D substrates. The 3D configurations of their DMF chips reduced cross contamination and improved the routing procedure for automation purposes. Li *et al.*<sup>81,82</sup> developed a multilayer 3D fabrication method to embed micro-heater and photodetectors in the planar DMF chip. They successfully implemented such a compact 3D configuration for on-chip polymerase chain reaction (PCR) applications.

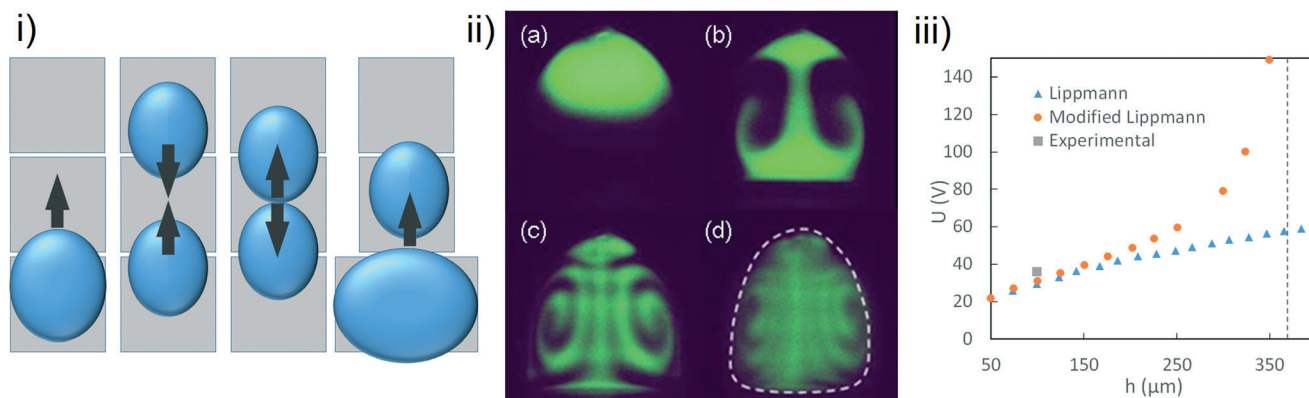
Digital microfluidics has also been interfaced with other microfluidics systems.<sup>83–87</sup> Such hybrid microfluidic devices provide the benefits of discrete format of DMF along with the advantages of continuous microfluidics for sample processing. To interface a pressurized flow within a microchannel with a sandwiched DMF system, Ahamed *et al.*<sup>83</sup> developed a thermal passive microvalve to control the delivery of the liquid sample from the microchannel into the DMF section of the chip. The microvalve functioned based on EWOD actuation of a thermo-responsive polymer for controlling the flow. Banerjee *et al.*<sup>86</sup> developed a programmable high throughput hybrid systems including micro-channels interfaced with a DMF platform. In order to achieve the high throughput sample processing platform, while benefiting from the advantages of DMF, parallel channels were fabricated. The device was programmed to generate droplets from the continuous flow through each channel. After processing each droplet, it was transferred and merged in to another channel. Shih *et al.*<sup>87</sup> interfaced a droplet microfluidic system with DMF for single cell applications. Their device uses the advantages of each system: the droplet microfluidic system is used to generate droplets which encapsulate single cells, and then the DMF system is used for highly controlled manipulation of the formed droplets.

### 3. Basic fluidic operations

Manipulation of droplets by EWOD began after the introduction of the technique by Berge<sup>88</sup> who added an insulating layer on the electrodes to separate them from the conductive liquid and prevent the electrolysis problems. A review of early studies on this phenomenon can be found in ref. 89; a more thorough review on EWOD droplet actuation can be found in ref. 37. As mentioned earlier, two categories of DMF devices consist in open and closed (sandwiched) systems. While access to the droplet (and sample delivery) is easier on the open system, the closed system allows droplet manipulation (transport and splitting) in more straightforward manner.<sup>22</sup> Four basic fluidic operations developed on DMF are droplet transport, mixing, splitting and dispensing (Fig. 2i) which enable the DMF devices for sample manipulation. Pollack *et al.*<sup>9</sup> demonstrated that EWOD can be used to transport aqueous droplets on the chip. Droplet splitting and mixing in silicone oil were later introduced.<sup>25</sup> The use of silicone oil facilitates droplet manipulation on the chip as the contact angle becomes larger and contact angle hysteresis smaller.<sup>14</sup> The four basic fluidic operations were performed and further studied with air as the surrounding medium by Cho *et al.*<sup>14</sup> Further improvements in the device fabrication resulted in manipulation of droplets with a voltage in the range of 20 V and a transport speed of several  $\text{cm s}^{-1}$ .<sup>37</sup> Use of an AC applied voltage rather than a DC voltage resulted in reducing the contact angle hysteresis and delaying dielectric breakdown.<sup>37,90</sup> A proper frequency of the applied voltage based on the system properties was also found based on the theory introduced in ref. 91 and 92. Their study showed that applying DC pulses rather than an AC (or DC) voltage can prolong the chip lifetime and increase the velocity of the droplet transport significantly.<sup>93</sup>

The primary technique for droplet mixing was moving the droplet back and forth on an array of electrodes which took several seconds.<sup>14</sup> Subsequently, more advanced techniques were developed for faster droplet mixing using enhanced EWOD manipulation of droplets by monitoring the liquid motion inside the droplet,<sup>94,95</sup> rotating the droplet over a 2D array of electrodes,<sup>96</sup> and frequency-dependent droplet oscillation<sup>97,98</sup> (Fig. 2ii), reducing significantly the mixing time.

Compared to droplet mixing and transport, droplet splitting and dispensing (from a larger mother droplet as a reservoir) are more challenging, as they require larger applied voltage and are limited to the geometrical parameters of the device.<sup>18</sup> Successful application of DMF for lab-on-a-chip applications depends on the precision of the device in sample preparation. Therefore, high control on the droplet size is required when droplet extraction or splitting is performed.<sup>18,19</sup> In general, droplet splitting is carried out on an array of 3 electrodes. The droplet is placed on the middle electrode, and by turning on the two side electrodes, the droplet will pinch and then split.<sup>14,99,100</sup> Droplet splitting depends on the gap height between the top and bottom plates, the size of the droplet and the electrodes, and the applied voltage.<sup>14</sup> Berthier *et al.*<sup>101,102</sup> developed an analytical model to determine



**Fig. 2** i) From left to right, schematic of droplet transport, merging, splitting and dispensing. ii) Fluorescence illustration of droplet mixing using frequency-dependent droplet oscillation (reproduced from ref. 97 with permission from American Institute of Physics). iii) Theoretical graph of the threshold splitting voltage versus the gap height (reproduced from ref. 102 with permission from Nano Science and Technology Institute).

the required voltage for droplet extraction from a reservoir as a function of the gap height. They showed that for a certain size of the electrodes, there is a limit for the gap height for droplet extraction (Fig. 2iii). In recent studies conducted on droplet splitting/dispensing, multiple approaches were attempted to reduce the error of the droplet size to less than 1%. Gong and Kim<sup>19</sup> developed a feedback control and capacitance measurement system (integrated into the DMF device) to control the volume of the extracted droplets by adjusting the applied voltage to the activation electrodes. Banerjee *et al.*<sup>103</sup> and Liu *et al.*<sup>20</sup> showed that using a continuous reservoir as well as ramping down the applied voltage to the middle electrode facilitates the generation of precise volumes of droplets. Samiei and Hoorfar<sup>18</sup> illustrated that applying a low voltage close to the threshold voltage required for splitting results in a split/dispensed droplet with a precise volume. They also showed that for specific electrode geometry, the relation between the threshold splitting voltage and the gap height is linear, which facilitates the selection of a proper voltage for precise splitting.

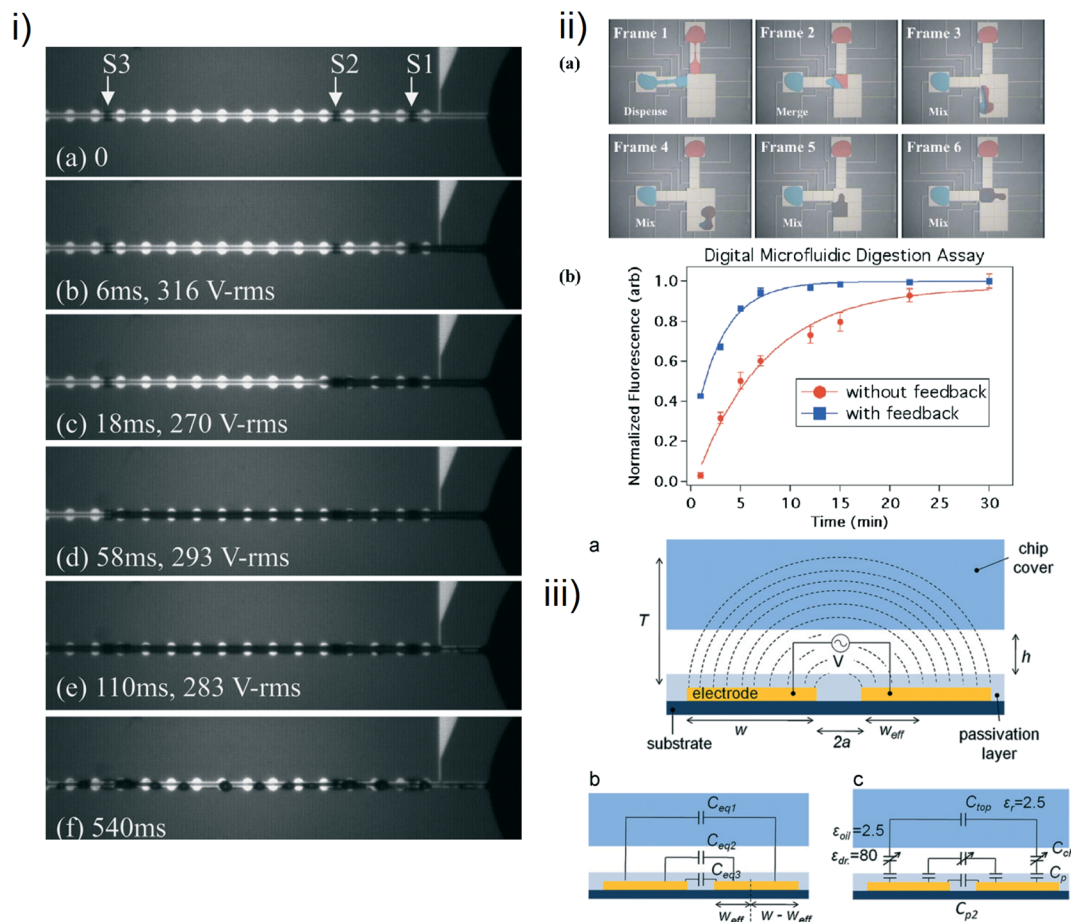
The developed techniques for precise and rapid droplet manipulation discussed so far are not limited to conductive and aqueous solutions. Manipulation of dielectric droplets,<sup>104</sup> organic and ionic solutions<sup>105</sup> and biological samples<sup>23,106</sup> has also been performed in numerous studies, illustrating the significant progress in sample manipulation on DMF systems.

## 4. Automation

Soon after the development of the basic fluidic operations and successful droplet handling on DMF devices, feedback control and sensing systems were integrated to these devices to manage the droplet formation and to automate on-chip processes. Image processing,<sup>107</sup> thin film<sup>108</sup> and optical fiber<sup>109</sup> based sensing systems have been used for controlling the droplet size and position (see Fig. 3i for droplet generation and Fig. 3ii for droplet size monitoring). However, most of the developed sensing systems for controlling the sample

manipulation of DMF devices have been based on capacitance measurement to determine the droplet size and interface position.<sup>17,110,111</sup> Multiple studies used the measurement of the change in the capacitance between the top and the bottom plates due to the presence of the droplet to automate sample manipulation.<sup>49,112,113</sup> Precise generation of droplet volumes<sup>19,20</sup> and control of the mixing process<sup>114</sup> have also been performed using these feedback control and sensing systems. In a different configuration, droplet sensing using the co-planar electrodes, *i.e.* using a pair of actuating electrodes on the bottom plate, has been demonstrated (Fig. 3iii) as an accurate way to monitor the droplet size and position.<sup>115–117</sup>

For parallelization on DMF devices, it is necessary to efficiently allocate sites on the chip for droplet storage and manipulation, and identify the motion paths for performing a large number of assay-related operations on a 2D array of electrodes. Böhringer<sup>16</sup> presented multiple algorithms for automating the parallel tasks performed on the chip. These algorithms include the task and motion planning for manipulation of several droplets simultaneously, as well as approaches to transfer the laboratory protocols to control commands compatible with DMF systems. Despite the general success of these algorithms in providing efficient solutions for performing parallel operations, they were not able to identify the optimum solution due to a high number of possible processes on a 2D array of electrodes. Taking advantage of the strategies used in very-large-scale-integration (VLSI) design for wire routing, Su *et al.*<sup>118</sup> developed a routing technique for droplet manipulation on DMF systems to minimize the number of electrodes used for the parallel processes to achieve a high throughput device. In this study the physical constraints imposed by the liquid and chip (such as minimum distance between droplets to avoid accidental coalescence) were also taken into account and the developed algorithm was tested experimentally. This method resulted in identifying the maximum number of parallel operations that could be achieved with a given number of electrodes. In a different study conducted on parallelization on DMF systems a



**Fig. 3** i) Droplet generation aided by optical feedback control (reproduced from ref. 109 with permission from The Royal Society of Chemistry). ii) (a) Sequences of an enzymatic assay, and (b) comparison of the outputs with and without feedback control (reproduced from ref. 113 with permission of The Royal Society of Chemistry). iii) Control of droplet size and transport speed by capacitive feedback control using coplanar electrodes (reproduced from ref. 117 with permission from Elsevier).

connect-5 structure of pin configuration was used to control the droplet paths to minimize cross-contamination between droplets.<sup>119</sup> This method can also identify the optimum solution for performing parallel operations. Application of such parallelization strategies have been shown for PCR<sup>82</sup> and cell-based<sup>79</sup> applications considering the cross contamination issues and routing optimization. Such advances in parallelism, along with the developed feedback control and sensing systems provide the capability of developing high throughput DMF-based platforms for performing multiple biochemical assays.

## 5. Manipulation of biological samples

When handling aqueous and non-biological samples, the hydrophobicity of the surface of the DMF devices prevents any residue of the samples on the chip and consequently there is no, or negligible cross contamination even in numerous cycles of droplet manipulation. On the other hand, handling biological samples leaves permanent traces on the hydrophobic surface of the chip, resulting in cross contamination and hindering further sample manipulation.<sup>70</sup> This is mainly

caused by hydrophobic adsorption and electrostatic interaction of the molecules such as proteins and lipids with the hydrophobic layer (deposited on the electrodes), which makes the surface permanently hydrophilic.

Multiple techniques have been developed to prevent or limit molecular adsorption to the surface of microfluidic devices with more or less success. In the first attempt, developed by Yoon and Garrell,<sup>120</sup> the pH of the sample, as well as the frequency, magnitude and the actuation period of the applied voltage were controlled to minimize the molecular adsorption on the surface. The use of silicone oil to insulate the sample droplet from the chip surface was another strategy used for EWOD actuation of body fluids such as whole blood.<sup>106</sup> This method was successful for transporting a blood droplet over thousands of cycles without surface contamination. However, sample contamination and subsequent detection of the targets can be a great concern.<sup>121</sup> Bayiati *et al.*<sup>122</sup> used plasma deposition technique instead of spin coating for the hydrophobic fluorocarbon deposition on the DMF and studied the decrease in the contact angle upon applying the voltage. A significant reduction of the surface contamination could be achieved with plasma deposition

method, after the solutions containing proteins were incubated on the device for 5 minutes. In another study, the protein adsorption onto the surface was investigated in the presence of a low concentration of Pluronic F127.<sup>70</sup> The sample observation by confocal microscopy and mass spectrometry allowed to conclude a reduced non-specific adsorption of proteins even though a concentration of protein as high as  $1 \text{ mg ml}^{-1}$  was used (Fig. 4i). The mechanism for such antifouling effect was hypothesized to be the formation of a Pluronic layer at the liquid phase boundary, similar to that reported for the use of silicone oil. Yang *et al.*<sup>123</sup> developed a strategy based on disposable polymer skins: after each experiment the top layer is removed and a new layer is replaced to eliminate cross-contamination and solve the device breakdown problems (Fig. 4ii). Despite the ease of this technique, it is not practical for point-of-care applications as it requires the manipulations to be performed in a cleanroom and the device should be assembled by well-trained personnel. Generating nanostructured super-hydrophobic surfaces is another method used for preventing surface biofouling.<sup>124,125</sup> The super-hydrophobicity of the surface significantly decreases adsorption of biomolecules to the surface, providing anti-biofouling properties. Prakash *et al.*<sup>125</sup> successfully used a DMF platform equipped with such a nanostructured super-hydrophobic surface for PCR detection of Influenza virus.

## 6. Advanced operations

Basic fluidic operations (droplet transport, mixing, splitting and dispensing) are the key to the functionality of the DMF devices for performing biochemical applications. However,

performing complete biological assays on such devices will not be feasible only by basic fluidic operations. The implementation of some strategies or integration of modules into the chip has to be considered for advanced operations or processes such as cell manipulation, particle binary separation, heating and biochemical sensing. For some operations such as particle manipulation, the DMF setup does not require reconfiguration. Only by modulating the applied voltage to the electrodes, the operations are carried out. Conversely, for multiple other advanced operations the geometry of the planar electrodes has to be modified or some external modules should be integrated according to the electrical, optical, and biochemical properties of the sample and the chip.

One of the advanced and essential operations is the ability to dispense variable volumes of samples or split a mother droplet into two unequal (volume) daughter droplets. This operation is important for particle and cell separation/concentration<sup>126,127</sup> or sample dilution<sup>47,106</sup> for which the use of equal droplet dispensing or splitting requires several repetitions, increasing significantly the process time and errors in the final product.<sup>18</sup> An example of a technique for unequal droplet splitting and dispensing variable volumes of droplets was demonstrated by Samiei and Hoorfar.<sup>18</sup> This technique works based on the geometrical modification of a conventional electrode on the DMF chips. The normalized size of the split droplets *versus* the number of actuated sub-electrodes (area of the actuated region) using this method is shown in Fig. 5i. In another study, voltage of different amplitudes was applied to dispense variable droplet volumes from a reservoir: the volume of the dispensed droplets was controlled by a capacitive sensing and feedback control system

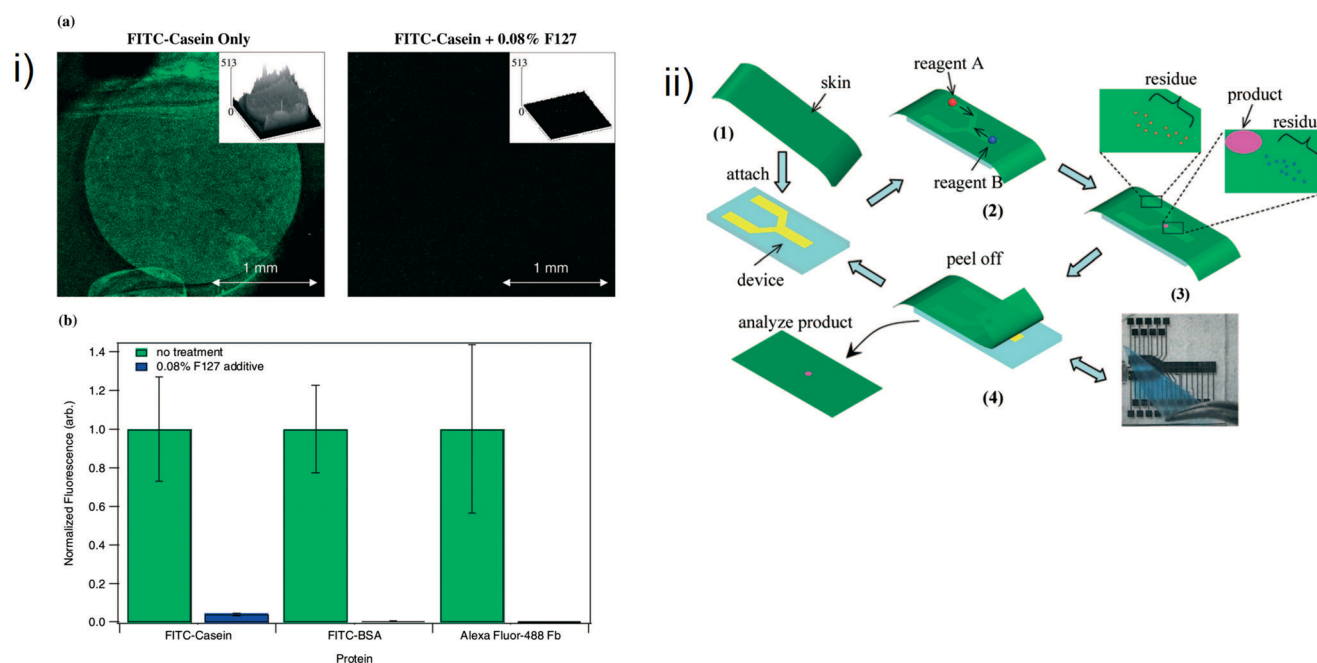
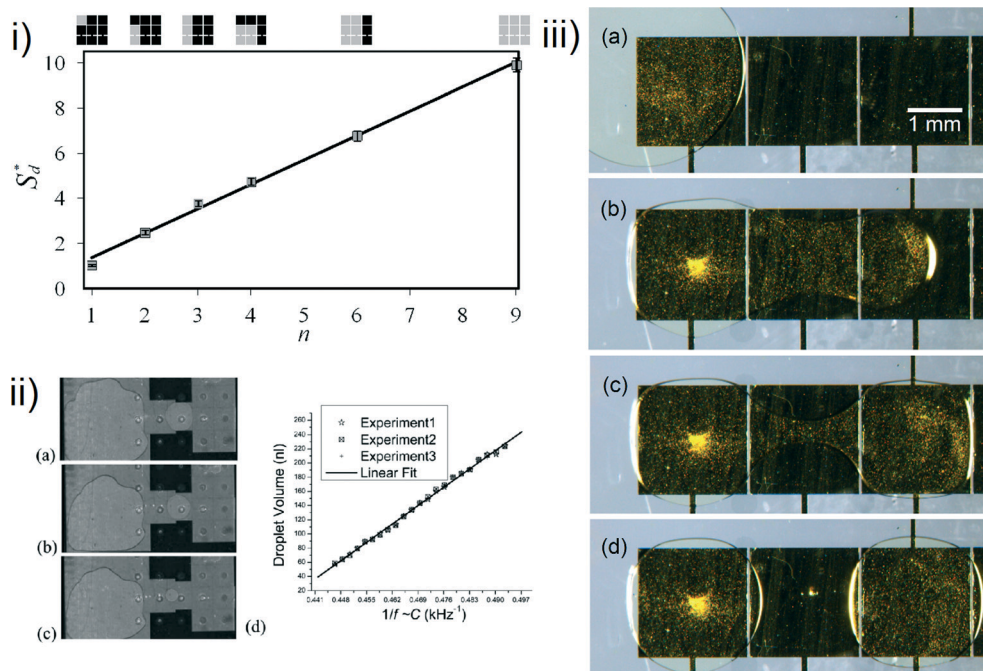


Fig. 4 i) Confocal microscopy images of protein adsorption onto the surface with and without using Pluronic F127 (reproduced from ref. 70 with permission from The American Chemical Society). ii) Disposable hydrophobic surfaces for DMF (reproduced from ref. 123 with permission from The American Chemical Society).





**Fig. 5** i) Unequal splitting by geometrical modification of a conventional DMF electrode. Normalized surface area of the split/dispensed droplet versus the number of the actuated sub-electrodes is shown to be linear (reproduced from ref. 18 with permission from Institute of Physics Publishing). ii) Feedback control of the droplet size by capacitive measurement (reproduced from ref. 19 with permission from The Royal Society of Chemistry). iii) Particle focusing using a dielectrophoretic-gravity driven (DGD) technique: (a) initial droplet, (b) focused particles using DGD, (c) and (d) splitting the droplet to two parts, one with a high and the other with a low concentration of the particles (reproduced from ref. 128 with permission from The American Institute of Physics).

(Fig. 5ii).<sup>19</sup> Both studies have reported an error of less than 1% for the volume of the dispensed droplets.

Manipulation of micro-particles inside the droplet has been performed in multiple studies for applications such as particle focusing (Fig. 5iii),<sup>128</sup> patterning<sup>129</sup> and separation.<sup>130,131</sup> These techniques have been mostly applied to microbeads which are important in several biological applications.<sup>132–135</sup> Similar strategies can be adopted for the manipulation of cells and biomolecules, such as optoelectric driven cell manipulation presented by Shah *et al.*<sup>136</sup> The particle manipulation techniques developed for DMF function based on optical,<sup>136</sup> magnetic,<sup>130,137–139</sup> hydrodynamic,<sup>140–142</sup> electrophoretic<sup>143</sup> and dielectrophoretic<sup>126,128,129,144,145</sup> properties of the particles and or the medium (droplet). For instance, using a dielectrophoretic-gravity driven (DGD) technique,<sup>128</sup> the particles could be focused on one side of the droplet, and then by splitting the droplet into two smaller droplets, two different concentrations (low and high) of particles with a focusing rate of over 90% were obtained (Fig. 5iii). Zhao *et al.*<sup>131</sup> used a traveling-wave dielectrophoretic technique to separate two types of particles by controlling the frequency of the applied voltage. Fig. 6i shows the sequences of concentrating magnetic beads by collecting them in one side of the droplet using an external magnet, and then splitting the droplet. This allowed for concentrating over 90% of the beads in one of the split droplets (Fig. 6i).<sup>130</sup> Focusing non-buoyant particles in a micro-droplet using a hydrodynamic-gravity driven method is illustrated in

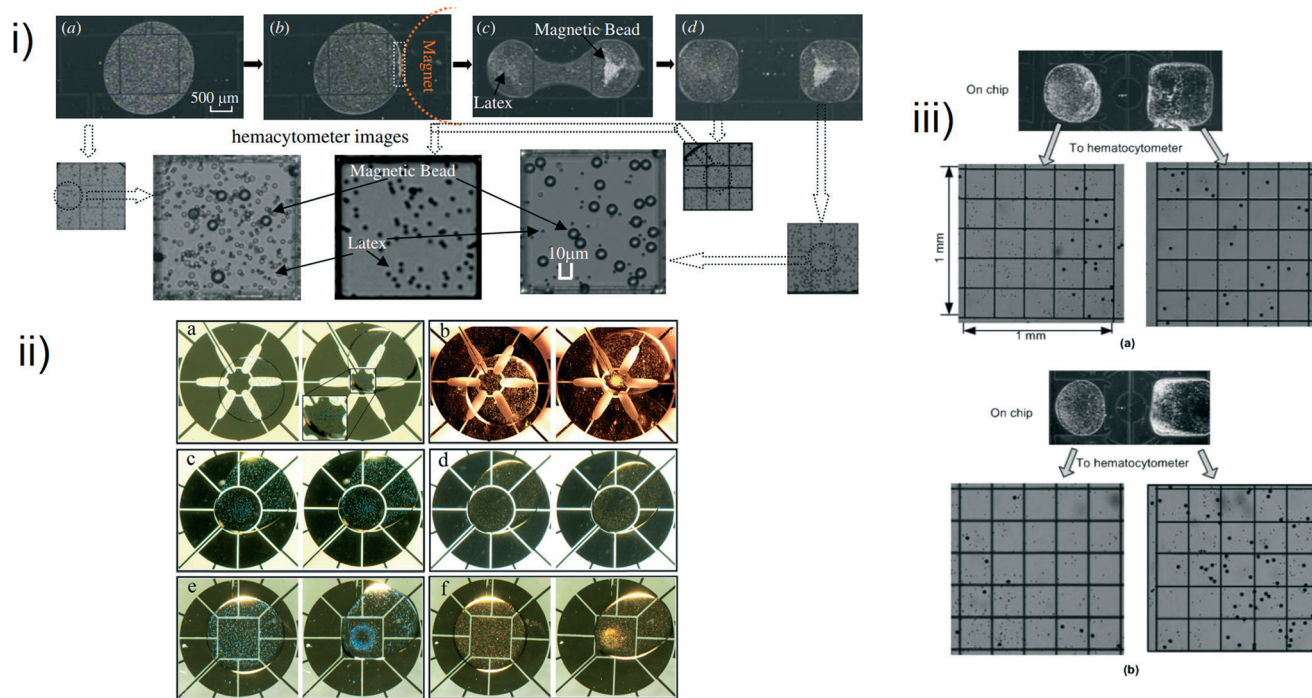
Fig. 6ii.<sup>140</sup> Electrophoretic separation of one type of particles along with the binary separation of two different types of particles with opposite charges was also performed (Fig. 6iii).<sup>143</sup>

Ionic liquids have been used as microreactors on DMF, in which such liquids are used as the soluble supports for performing solution-phase synthesis.<sup>146</sup> The stability of these liquids due to their low level of volatility makes them a proper reactor for chemical applications in open systems. The basic fluidic operations (transport, mixing, splitting and dispensing) have been performed on micro air bubbles similar to those for droplets.<sup>147</sup> This can provide a powerful tool for gas analysis on DMF platforms. Most recently, a new simple method was developed<sup>148</sup> to prevent evaporation problems on an open DMF system. This method was based on just-in-time replenishment of the solvents to keep their volume large enough for the biochemical assay.

Sista *et al.*<sup>149</sup> and Li *et al.*<sup>150</sup> reported the integration of a heater to the DMF platform for the thermal control of the assays. The heaters are patterned on the chip similar to the method used for electrode fabrication. In essence, a thermal element is formed by a metal layer, and heat is generated by passing a controlled electrical current through the electrode.

## 7. Detection

Sensing or detecting a biological event on the chip is an important and inseparable part of any biochemical assay. Numerous investigations are dedicated to develop detection



**Fig. 6** i) Magnetic separation of particles inside the droplet followed by splitting, resulting in two daughter droplets with high concentration of different particles (reproduced from ref. 130 with permission from Institute of Physics Publishing). ii) Gravity-driven hydrodynamic focusing of non-buoyant particles (reproduced from ref. 140 with permission from The Royal Society of Chemistry). iii) Binary separation of particles using electrophoresis (reproduced from ref. 143 with permission from The Royal Society of Chemistry).

systems being used with DMF platforms. A review of detection on DMF systems was performed by Malic *et al.*<sup>46</sup> While the majority of the approaches are based on fluorescent microscopy,<sup>151</sup> other techniques such as mass spectrometry, chemiluminescence and electrochemical systems have also been used for detection of targets on DMF. As such, these methods can be categorized in optical, electrochemical and mass spectrometry according to their sensing mechanisms. They can also be classified on their mode of detection, *i.e.* off-line or off-chip and on-line or on-chip detection.<sup>46</sup> While the off-line mode is suitable for in-lab uses, on-line systems are beneficial for packed and portable devices for point-of-care applications.

Regardless of the transduction mechanism (electrical, electrochemical, optical and mechanical), all biosensors require the modification of their sensing surface by functionalizing it with selective bio-receptors as biological recognition elements. Based on the surface modification techniques, biosensors can be categorized<sup>152</sup> as enzyme-based,<sup>153–155</sup> antibody-based,<sup>156,157</sup> and aptamer-based<sup>158–160</sup> biosensors. A review of different surface modification techniques for biosensors integrated into microfluidic devices can be found in ref. 152. Implementing similar techniques for surface functionalization, many biosensors have been developed for DMF devices which are covered in this section.

Off-line detection based on optical methods<sup>161</sup> (such as fluorescence,<sup>123,162,163</sup> absorbance,<sup>137,146</sup> and chemiluminescence<sup>137</sup>) along with mass spectrometry<sup>164–166</sup> are major sens-

ing techniques to analyze samples extracted from DMF platforms. Label-free optical detection such as surface plasmon resonance (SPR)<sup>167,168</sup> has also been used. The reason behind the popularity of the off-line methods is that the required equipment is readily available in most of laboratories for biochemical or cell-based assays.<sup>47,58,169</sup>

As for the online detection, the conventional design and configuration of the detection systems have to be modified to miniaturize them for integration into the DMF devices. Examples are fluorescence detection for the polymerase chain reaction (PCR),<sup>149,170</sup> proteomics<sup>171</sup> and enzymatic assays.<sup>169</sup> Other optical methods such as chemiluminescence<sup>108,137,149,172</sup> and absorbance<sup>59,106</sup> have also been integrated into DMF devices. The requirement for this integration is to leave the top or the bottom plate transparent (*e.g.* the use of ITO as the electrode) in order to have optical access to the sample. For instance, Fig. 7i shows a metal-semiconductor-metal (MSM) photodetector fabricated on the top plate of a DMF chip for chemiluminescence sensing.<sup>108</sup> Fig. 7ii illustrates the schematic of an absorbance-based optical biosensor for the glucose assay integrated into a DMF platform.<sup>106</sup> The photodiode was placed on the surface of the top plate and the electrodes were fabricated using ITO to allow the transmission of light from underneath the chip towards the top plate. An electrochemiluminescence technique was used on a DMF device for the detection of single nucleotide mismatches of microRNA-143 (miRNA-143) in cancer cell lysates with a high sensitivity.<sup>173</sup> Similarly, miniaturized SPR

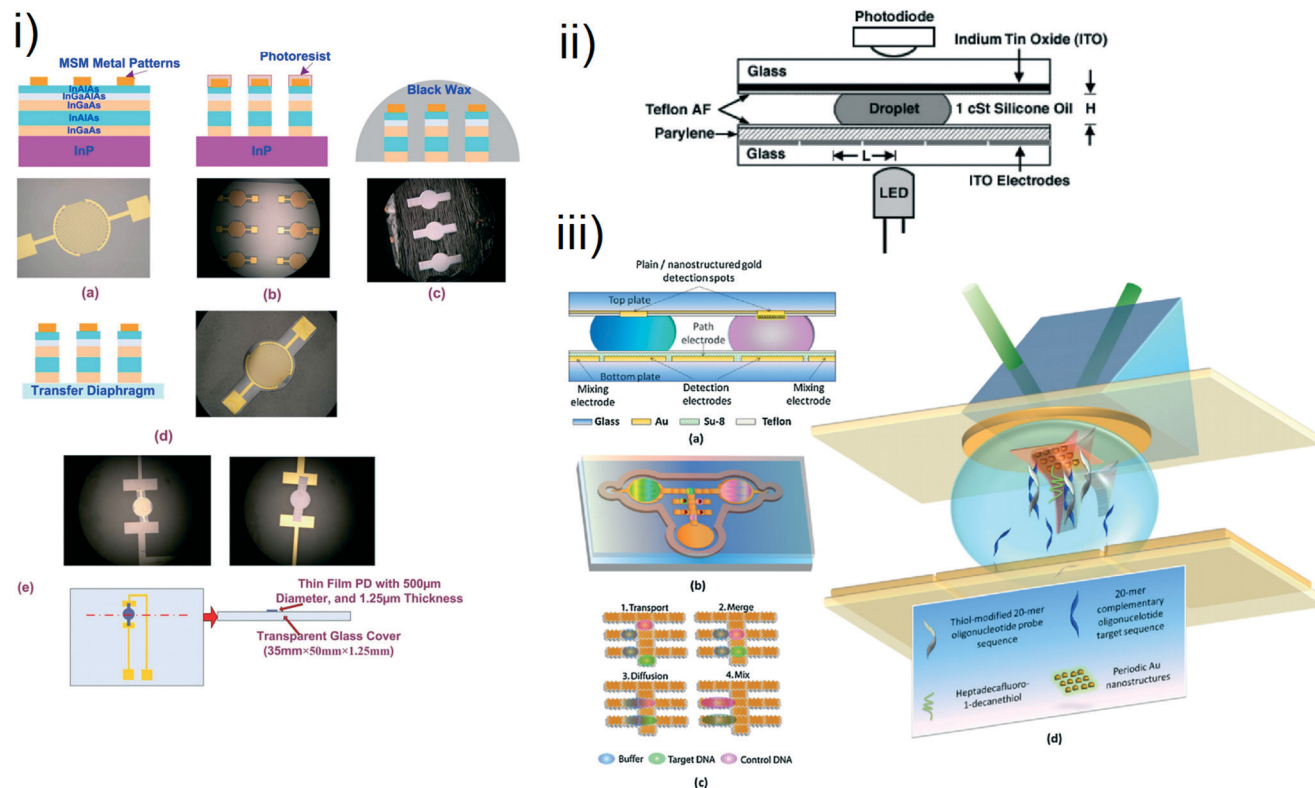


Fig. 7 i) Sequences of fabrication and on-chip integration of a metal-semiconductor-metal (MSM) photodetector for chemiluminescence detection (reproduced from ref. 108 with permission from IEEE). ii) Schematic of an optical absorbance detector integrated into a DMF chip (reproduced from ref. 106 with permission from The Royal Society of Chemistry). iii) Schematic of a SPR biosensor integrated into a DMF chip (reproduced from ref. 176 with permission from Elsevier).

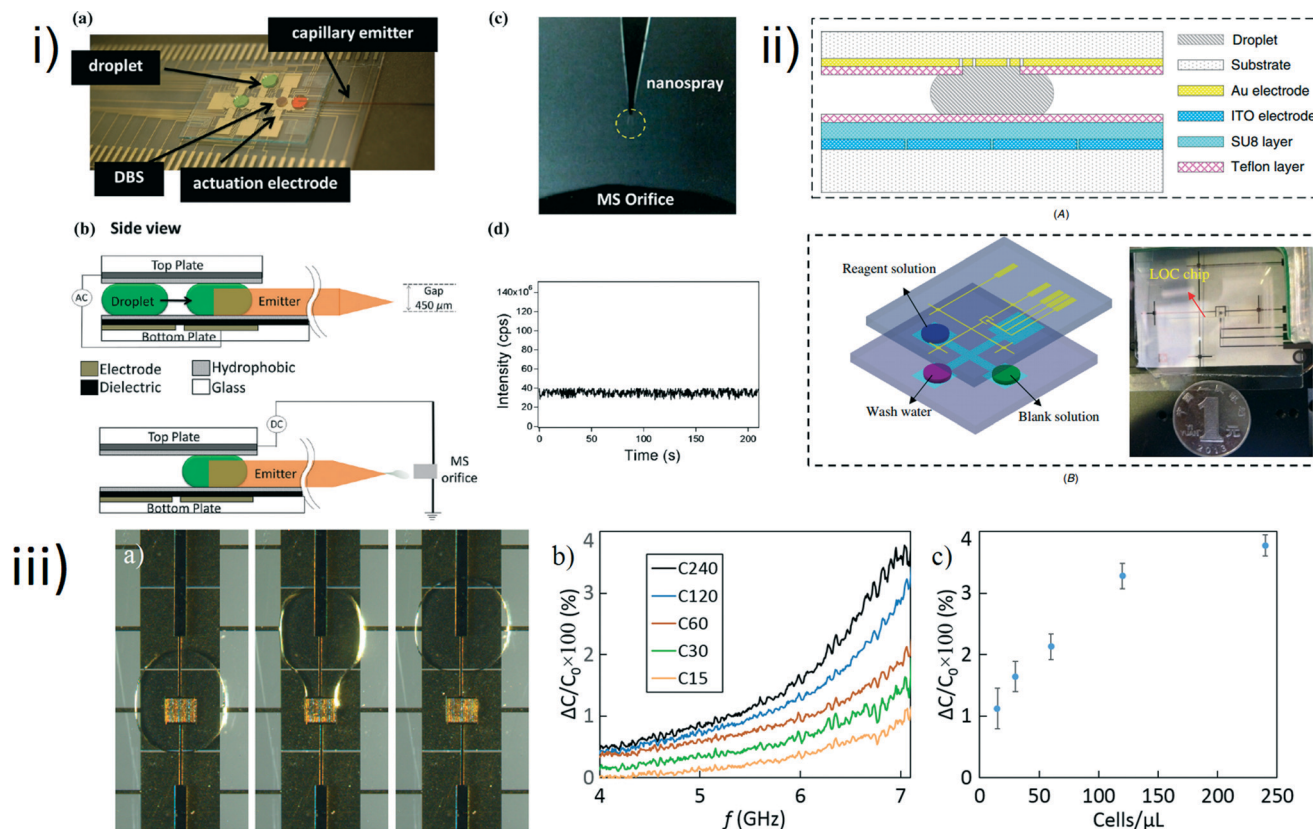
(Fig. 7iii) biosensors have also been integrated into DMF.<sup>174–176</sup> A detection limit as low as 5 pM was achieved for the DNA hybridization.<sup>176</sup> Multiple studies report on the integration of mass spectrometry detection system into DMF.<sup>177–180</sup> Fig. 8i shows a nano electrospray ionization-mass spectrometry system interfaced with a DMF chip used for the analysis of dried blood spots (DBSs).<sup>178</sup> Interfacing between DMF and liquid chromatography-mass spectrometry has further been demonstrated in ref. 181. A major issue with such systems is that they cannot be miniaturized for complete integration into DMF, and hence the on-line detection mode has been performed by interfacing these systems to DMF for real time detection.<sup>46</sup> Unlike the optical and mass spectrometry detection systems which require external devices (light source, photo detector *etc.*) to be integrated into the chip, the electrochemical sensing systems can be patterned and directly integrated on the DMF chip along with the actuating electrodes with a very easy and straightforward fabrication method.<sup>46,146,182,183</sup> An example of such systems is shown in Fig. 8ii in which the three-electrode sensor is used for the detection of different concentrations (down to 500 nM) of ferrocenemethanol (FcM) and dopamine (DA).<sup>182</sup>

One main problem associated with the integrated biosensors into DMF is the hydrophilic nature of their sensing surface, which hinders droplet actuation. Multiple studies addressed such a problem by limiting the size of the sensing

surface to minimize the amount of the sample liquid left on the biosensor.<sup>174,182,184</sup> Most recently, the study by Samiei *et al.*<sup>156</sup> highlights the effects of geometrical configurations of the biosensors and the DMF platform on droplet manipulation to achieve the largest possible surface area of the biosensor for which droplet removal is performed after sensing. According to this study, the biosensor (fabricated on the top plate) is recommended to be patterned with a high aspect ratio and aligned with the center of the actuating electrode on the bottom plate. Also, the gap height between the two plates should be set to the upper limit of the range which allows for precise droplet manipulation. Fig. 8iii illustrates the sequences of droplet removal after sensing, along with their results of the capacitive detection of different concentrations of *Cryptosporidium*.

Other than the optical, mass spectrometry and electrochemical detection methods, hydrodynamic properties of the sample has also been used for detection.<sup>140</sup> Using the electrode configuration shown in Fig. 9i, a sample of polystyrene beads could be concentrated after spinning the droplet for a certain period. However, when a small concentration of DNA (18 ng  $\mu\text{L}^{-1}$ ) was added to the sample, the focusing behavior was different and the microbeads left the central region of the electrode design.

Recently, integration of a field effect transistor (FET) biosensor into DMF was performed. Results showed that the



**Fig. 8** i) Interfacing between a DMF setup and nano electrospray ionization-mass spectrometry using a capillary emitter (reproduced from ref. 178 with permission from The American Chemistry Society). ii) Integrated electrochemical detection electrodes fabricated on the top plate of a DMF setup (reproduced from ref. 182 with permission from Institute of Physics Publishing). iii) Sequences of complete droplet removal after sensing, along with the results of the capacitive detection of different concentrations of Cryptosporidium (reproduced from ref. 156 with permission from Elsevier).

FET biosensor is a highly selective and sensitive on-chip detection method for such an ultra-small scale (Fig. 9ii).<sup>157</sup> The high selectivity and sensitivity, along with the miniaturized geometry are the features that are pursued for the development of many label-free nano-biosensors, particularly for electrical- and optical-based sensors. The ultra-small size of these sensors along with the use of biological receptors immobilized on their surface make them very attractive for the fabrication of an array of sensors<sup>185</sup> for multiplexing and for integration onto DMF. A review of the label-free biosensors can be found in ref. 186.

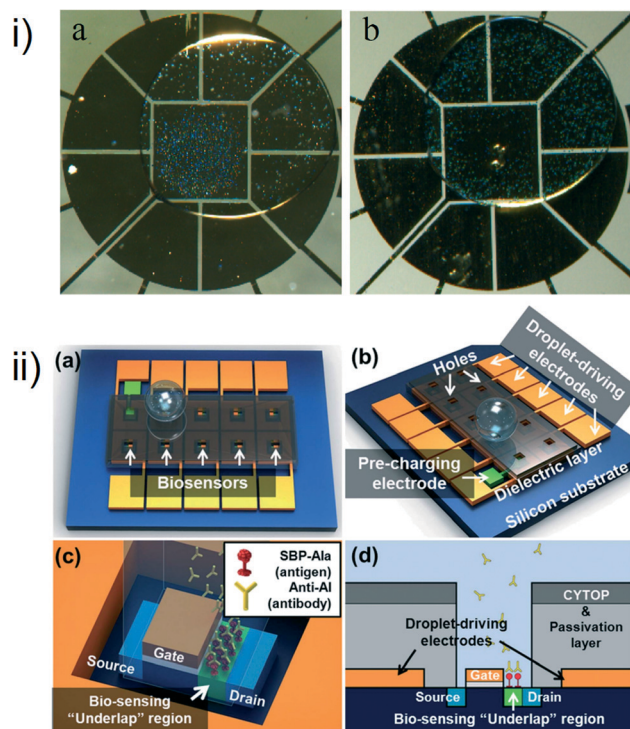
## 8. Biological applications

Since the introduction of DMF, the remarkable functionality of these devices and their high controllability regarding sample manipulation motivated the development of devices compatible with various biological applications.<sup>11,12,23,42–44,46,121,152,187,188</sup> In general, these applications can be categorized as clinical diagnostics applications, enzymatic assays, tissue engineering and cell-based applications, DNA-based applications, immunoassays and proteomics.<sup>11,46</sup>

For the clinical diagnostic applications, DMF was employed in some studies only for partial sample preparation

(e.g. for detecting mycoplasma DNA<sup>189</sup>), and in several studies, it was further developed for performing a larger portion of the diagnostic assay for lysosomal storage diseases,<sup>190,191</sup> dried blood spot (DBS) analysis<sup>192</sup> and estradiol detection.<sup>193</sup> For instance, Fig. 10i shows a device developed for extraction of samples from a dried blood spot (DBS) for amino acid quantification by tandem mass spectrometry.<sup>192</sup> Such devices can be used for a wider range of analyses on newborn DBSs. In the study conducted by Ng *et al.*, a complete sample preparation was performed on the chip for the detection of rubella infection and immunity, and the chip was placed under a chemiluminescence device for detection.<sup>194</sup> For this purpose, rubella virus immobilized on the magnetic beads were used for capturing the analyte from the sample. Specific antibodies with linked enzymes were used for generating the chemiluminescence signal upon antibody-analyte binding. Most recently, Millington *et al.* developed a DMF package for multiple detection from the extracts of DBS for point-of-care applications.<sup>195</sup> In this study, the entire sample preparation and detection processes were performed on the DMF using colorimetric and fluorometric techniques.

DMF has widely been employed for chemical and enzymatic assays for analysis of the reaction kinetics and developing new compounds.<sup>11</sup> The notable enzymatic assays were



**Fig. 9** i) Detection of DNA in the sample by monitoring the change in the focusing behavior of the polystyrene microbeads after spinning on the electrode configuration for 250 s (reproduced from ref. 140 with permission from The Royal Society of Chemistry). ii) Schematic of an integrated FET biosensor into a DMF platform (reproduced from ref. 157 with permission from The Royal Society of Chemistry).

performed for glucose detection,<sup>59</sup> environmental applications,<sup>121,196</sup> diagnostics,<sup>197</sup> studying the kinetics of reactions,<sup>169,177</sup> synthesis of new compounds,<sup>146,198,199</sup> and for chemical synthesis.<sup>200,201</sup> For instance, Fig. 10i illustrates the sequences of an assay for quantification of glucose on a DMF device using an enzyme-kinetic based colorimetric method.<sup>59</sup>

Several studies have been conducted for DNA-based assays such as DNA purification,<sup>202,203</sup> detection of DNA hybridization,<sup>174,176,204</sup> DNA sequencing,<sup>205</sup> and PCR.<sup>150,163,206</sup> Fig. 10iii shows partial sequences of the extraction of genomic DNA from whole blood using magnetic beads. The procedure required however the use of magnetic beads and multiple washing cycles for purification of the extracted DNA.<sup>203</sup>

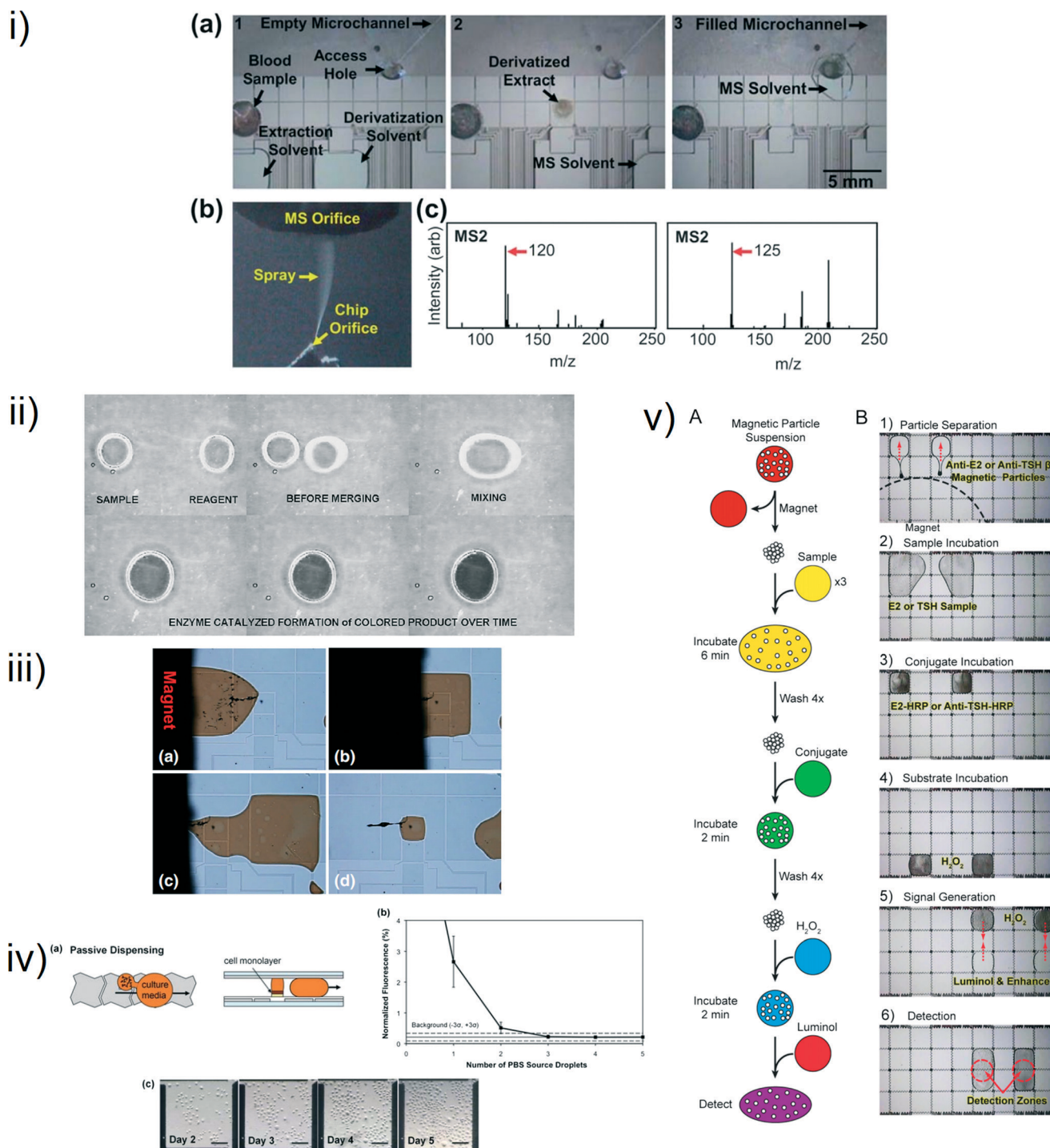
Application of DMF in tissue engineering and cell-based assays has been reported in multiple studies.<sup>44,45,58,207–213</sup> Fig. 10iv shows the application of DMF technology for cell culture in which a DMF device is developed with the capability of performing a complete set of processes for mammalian cell culture.<sup>207</sup> A DMF device was developed by Kumar *et al.*, with a microwell array for trapping single non-adherent yeast cells in femtoliter droplets which was used for cytotoxicity assays.<sup>210</sup> Bender *et al.*<sup>214</sup> used DMF for performing cell invasion studies for spheroid-based invasion assays. They showed human fibroblast spheroids invade collagen gels, and one can improve or hinder their invasion by delivering exogenous migration modulating agents.

Immunoassays have been extensively performed using DMF systems.<sup>137,160,215–219</sup> Fig. 10v illustrates the steps of multiple particle-based immunoassays performed on a DMF platform. The assay includes parallel 17 $\beta$ -estradiol (E2) and thyroid stimulating hormone (TSH) immunoassays using magnetic beads followed by chemiluminescence detection of the analytes using a well-plate reader.<sup>217</sup>

DMF has also been widely used for proteomics.<sup>47,171,219–225</sup> Luk *et al.*<sup>222</sup> used enzyme immobilized hydrogels patterned on a DMF chip as microreactors for proteolytic digestion. In this study cylindrical agarose discs were used as a platform for immobilizing trypsin and pepsin used for proteomic digestion, and mass spectrometry was used to analyze the assay products. Mok *et al.*<sup>225</sup> developed a DMF platform for protein biomarker detection for quantifying protein abundance and activity. In this study, interleukin-6 abundance and Abelson tyrosine kinase activity were quantified with a detection limit as low as 50 pM for interleukin-6 and 100 pM for kinase.

## 9. Packaging and portability

The devices developed by Gong *et al.*<sup>49</sup> and Gong and Kim<sup>15</sup> are likely the earliest attempts for a portable DMF package. A 2D array of electrodes was fabricated using a multi-layer printed circuit board (PCB) and used land grid array sockets for packaging the system. Although they performed multiple fluidic operations, their device lacked a detection system required for biological applications. Sista *et al.*<sup>149</sup> fabricated a portable DMF platform, capable of performing immunoassays, enzymatic assays and DNA amplification. The device had a PCB chips (Fig. 11i) and integrated reservoirs for sample delivery using a pipette as well as waste buffer removal. Magnetic and heating units required for performing the assays were embedded in the chip deck. Optical detection was integrated using chemiluminescence detection and fluorimetry units. Finally, an electrical controller unit was integrated to the device for controlling the on-chip processes. This study showed a successful attempt in developing a DMF based portable package for point-of-care applications (Fig. 11ii). Similarly, Kim *et al.*<sup>226</sup> fabricated a DMF hub for DNA applications with a capillary-based interface for straightforward sample delivery to and from the hub (Fig. 10iii). Most recently, Millington *et al.*<sup>195</sup> reported a portable device with the capability of performing different biomarker assays on the extracts of a DBS. In another study, a smart phone-controlled portable DMF platform was developed, in which open droplet manipulation system was used for sample processing and the detection was performed *via* a colorimetric method using the images captured by the smart phone.<sup>227</sup> Most recently, a prototype of a smart-phone-controlled low-cost portable DMF platform was fabricated with integrated chemiluminescence detection system.<sup>228</sup> The device was controlled using a DIY-built application, enabling droplet manipulation as well as on-chip chemiluminescence detection using a smart phone.

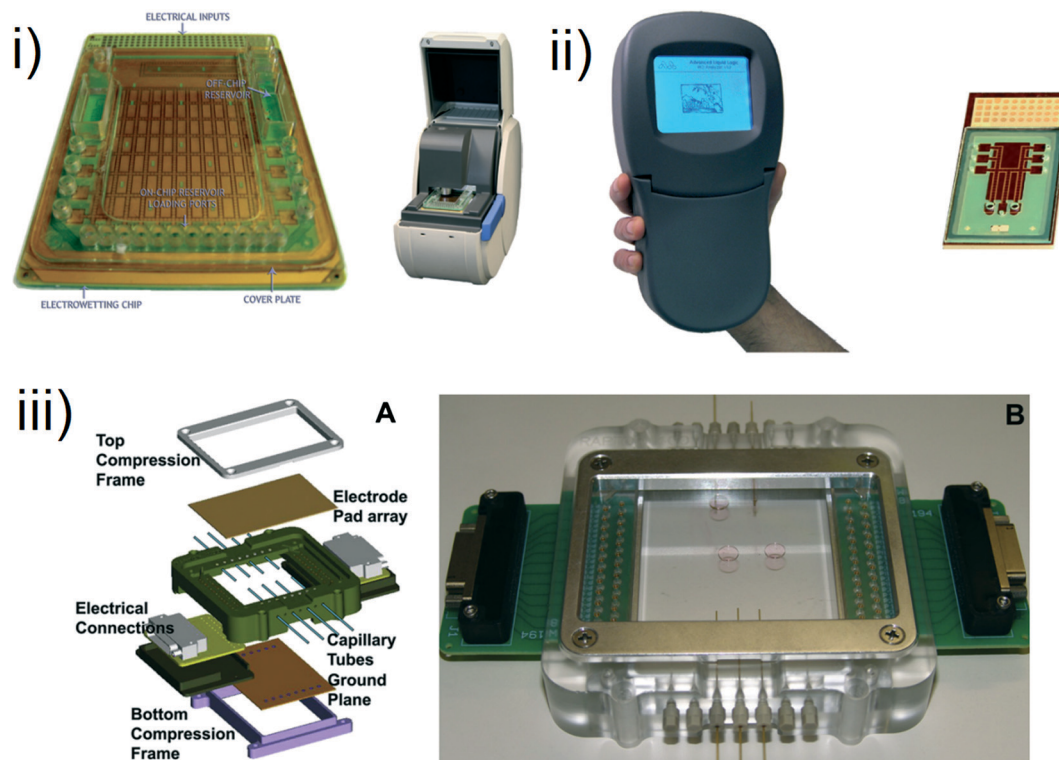


**Fig. 10** i) On-chip sample preparation and detection of amino acids in dried blood spots (DBSs) using mass spectrometry (reproduced from ref. 192 with permission from The Royal Society of Chemistry). ii) Sequences of an enzymatic assay for glucose detection (reproduced from ref. 59 with permission from Elsevier). iii) Sequences of genomic DNA extraction and purification from the whole blood using magnetic beads (reproduced from ref. 203 with permission from Springer). iv) A DMF chip for mammalian cell culture (reproduced from ref. 207 with permission from The Royal Society of Chemistry). v) Sequences of a particle-based immunoassay on a DMF platform (reproduced from ref. 217 with permission from The American Chemical Society).

## 10. Discussion and future trends

After reviewing the main recent progresses and advances in the development of DMF technology, the development of a

'portable' DMF platform capable of performing the entire assay processes (from sampling to sensing) is still under question. Certainly, for applications such as clinical diagnostics, enzymatic assays and immunoassays, this goal is achievable



**Fig. 11** i) Fabrication of a DMF multiwell plate using a PCB (reproduced from ref. 149 with permission from The Royal Society of Chemistry). ii) Development of a portable DMF package for performing immunoassays, enzymatic assays and DNA amplification (reproduced from ref. 149 with permission from The Royal Society of Chemistry). iii) Development of a DMF hub for DNA applications with capillary-based interface for sample delivery (reproduced from ref. 226 with permission from Sage Publications).

but certain obstacles in the areas of biological sample manipulation, detection, and packaging and portability must be overcome. The aspects related to fabrication, sample manipulation, and automation have been well advanced. For instance, high-resolution chips with the electrodes as small as  $21\ \mu\text{m}$ , capable of droplet formation with a volume as small as  $5\ \text{pL}$  have been reported in ref. 68. Other robust fabrication techniques such as micro-contact printing<sup>229</sup> have also been well developed for patterning different substrates. Conversely, multilayer chip fabrication has provided a large number of electrodes in a 2D array resulting in a high throughput platform.<sup>71,72</sup> Droplet manipulation is also performed with a high accuracy of the sample volumes.<sup>18</sup> Significant advances have been made in regards to droplet sensing and control algorithms permitting full automation and parallelization on DMF devices.<sup>16</sup>

Manipulation of biological samples on the chip has been the subject of many studies. However, developing self-cleaning surfaces with high durability and low of cross-contamination is not achieved yet. Examples of efforts made in this regard include the change in the pH of the solution along with optimizing the frequency and amplitude of the applied voltage,<sup>120</sup> the use of plasma deposited fluorocarbon<sup>122</sup> and the application of disposable polymer films.<sup>123</sup> However, neither of these methods can be used for development of permanent devices. For the long-term and especially for point-of-care applications, silicone oil<sup>106</sup> and Pluronic additives<sup>70</sup>

can be used. However, the suitability of these approaches are questionable due to sample contamination and limited choice of the detection method as they form a layer at the liquid boundary. In addition to these drawbacks, the durability of the device after several cycles of operation and compatibility with some biochemical samples are yet to be proved for Pluronic additives. Nano-structured super-hydrophobic surfaces have also been developed for self-cleaning purposes on DMF<sup>124</sup> However, durability of the surface after several cycles of manipulation of biological samples must still be studied. Other strategies developed for self-cleaning on non-DMF systems<sup>230,231</sup> have the potential to be used for DMF-based devices. Nevertheless, these methods need to be studied in terms of compatibility with DMF.

The on-line detection systems integrated into DMF platforms revealed as an essential step for portable DMF systems. The recently developed miniaturized SPR and electrochemical biosensors that are quite practical for a large number of detection cases<sup>176,232</sup> along with the FET biosensors are excellent choices for integration into DMF devices.<sup>185</sup> The main challenge still is the biological regeneration of the sensor by recovery of the receptors immobilized on the biosensors. Despite an incremental success made toward the regeneration of biosensors for non-DMF applications,<sup>233</sup> developing strategies and protocols for the recovery of the receptors compatible with the DMF platforms needs to be further investigated.

Finally, portable DMF platforms capable of performing one or a series of biochemical assays<sup>149</sup> can be developed by the aid of multilayer chips with integrated controllers for parallel sample processing and miniaturized operators such as detection systems and microheaters. Availability of electrical circuits for generating high AC voltage from a low voltage battery<sup>227</sup> and interfacing techniques for sample delivery to the device<sup>226</sup> are the critical features to be considered for the packaging aspect of DMF devices. Advances in 3D printing technology<sup>234</sup> has made a new paradigm for cost-effective packaging using different materials.<sup>235</sup> This technology can readily be used for packaging DMF-based LOC platforms for the development of portable devices for point-of-care applications.

## 11. Conclusions

Remarkable progress has been achieved towards developing multifunctional lab-on-a-chip packages capable of performing complete sets of processes required for biochemical assays using DMF technology. Numerous studies showed that fine electrodes and dielectric features can be fabricated with expected performance using the advanced microfabrication technologies. Droplets of pico-liter to microliter sizes can be created, transported and split with very high accuracy and in an automated and parallel fashion. Multiple techniques have been developed to reduce the rate of molecular binding to the surface for the manipulation of biological samples. Taking advantage of these features, numerous biological assays have been performed in DMF platforms. Similarly, multiple operating procedures have been developed for particle and cell manipulation, on-chip heating, unequal droplet splitting, sensing *etc.* To achieve reliable, multifunctional and portable DMF-based packages for point-of-care applications further improvements such as the development of a more robust surface coating are required to eliminate the problem of molecular binding and make the system reusable. The current methods such as the use of oil and Pluronic, or plasma deposition of fluorocarbons are not promising for long-term use. Another critical aspect is the development of an array of sensors for multifunctional and accurate on-chip sensing in DMF set-up. Recovery of the detection system is crucial for frequent uses of the device. Thus, the success in developing the desired DMF packages for long term use with the capability of performing a complete set of processes for multiple applications requires addressing limitations of the current technologies underlined in this review.

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