Lab on a Chip

TUTORIAL REVIEW

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Cite this: *Lab Chip*, 2025, **25**, 3297

Mammalian cell culture and analysis in digital microfluidic platforms

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Digital microfluidics has emerged as a promising platform for cell culture, offering precise control over droplet-based microenvironments while enabling automation and miniaturization. This review examines the integration of DMF with various cell culture substrates, including hydrogels and synthetic polymers, and evaluates their advantages and limitations for supporting cellular growth, adhesion, and viability. The influence of AC and DC actuation modes on droplet handling, as well as their effects on cell behavior, are also discussed. I elaborate how DMF systems can be designed to deliver and modulate physical and biochemical stimuli, such as shear stress, temperature, and gradients of signaling molecules, to better mimic *in vivo* conditions and study cell behavior in dynamic environments. Next, I highlight the incorporation of analytical tools and sorting techniques in DMF-based cell culture and explore its potential applications in organ-on-chip systems. While DMF presents unique opportunities for cell-based research, challenges such as material compatibility, cell viability, and system stability remain. This review provides a critical assessment of current developments and future directions for DMF in cell culture applications.

Received 10th March 2025,
Accepted 11th June 2025

DOI: 10.1039/d5lc00250h

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1. Introduction

Digital microfluidics (DMFs) is widely used in various applications in different fields ranging from environmental monitoring to inkjet printing.[1,2] In analytical chemistry, DMF facilitates automated assays or basic synthesis, reduces the consumption of reagents, and improves reproducibility.[3] In electronics manufacturing, DMF is used for the production of flexible circuits and micro-strips.[4] Such a broad versatility stems from DMF's ability to precisely process droplets in an automated manner. This versatility and potential can also be applied to biomedical engineering, especially in areas such as cell culturing, drug screening, antibody production, and point-of-care diagnosis, where precise microenvironment control and automation are also required. In the last decade, DMF has emerged as a functional and transformative tool for biological assays and is emerging further to expand its applications in cell culturing in order to boost the progress in biomedical engineering research.

DMF is a liquid handling method that uses arrays of microelectrodes to manage sub-microscale liquid manipulation semi-automatically and precisely, one drop at a time. Without the dead volumes of reagents, pumps, or actual microchannels, highly controlled and customizable conditions can be manipulated because of the ability to control individual droplets. It is important as it makes experiments more precise, which improves reproducibility and makes it easier to draw solid scientific results.[5] Furthermore, in recent years, several biosensors have been integrated into DMF systems, increasing the role of DMF in biomedical and cell culture research.[5] Such an integration is intended to guide the workflow from cell culture to sorting and analysis, providing a comprehensive approach that encompasses all aspects of cellular behavior study (Table 1).

In terms of cell culture experiments, DMF has various advantages over typical enclosed microfluidic chips (Fig. 1). One key advantage is the capacity to pinpoint and manipulate specific chemicals and cells. This feature eliminates the possibility of cross-contamination between the sterile and cell-seeded sides, as well as between old and new generations of cells, maintaining the integrity of experiments while such experiments can be performed using AI conveniently due to the typical computer-controlled droplet manipulation in DMF devices.[6] Furthermore, DMF enhances passive media exchange, reducing cell disruption and facilitating optimum cell adhesion to the surface.[7,8] Next, the use of sub-microliter volume droplets reduce reagent use while matching the scale of an experiment to the size of cells, allowing for precise and efficient cell handling. This property makes DMF an invaluable tool for studying smaller cell populations, and for examining cell heterogeneity within

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Table 1 Comparison between advantages and disadvantages of the available research models for cell culturing

Research model	Advantages	Disadvantages
Traditional 2D cell culture	Simple, cost-effective, well-established	Lacks physiological relevance, limited cell–cell interactions
3D cell culture (*e.g.*, spheroids, organoids)	Better mimics *in vivo* conditions, improved cell signaling	More complex, higher costs, variability in results
Microfluidic chips	Precise control over microenvironment, enables real-time monitoring	Requires complex connections, specific equipment such as pumps, valves, complex fabrication
DMF	Automated handling, low reagent consumption, scalable, enables high-throughput screening	So far limited adoption in biology, requires expertise in microfabrication and programming
Animal models	Physiological relevance, whole-organism interactions	Ethical concerns, high costs, time-consuming, species differences

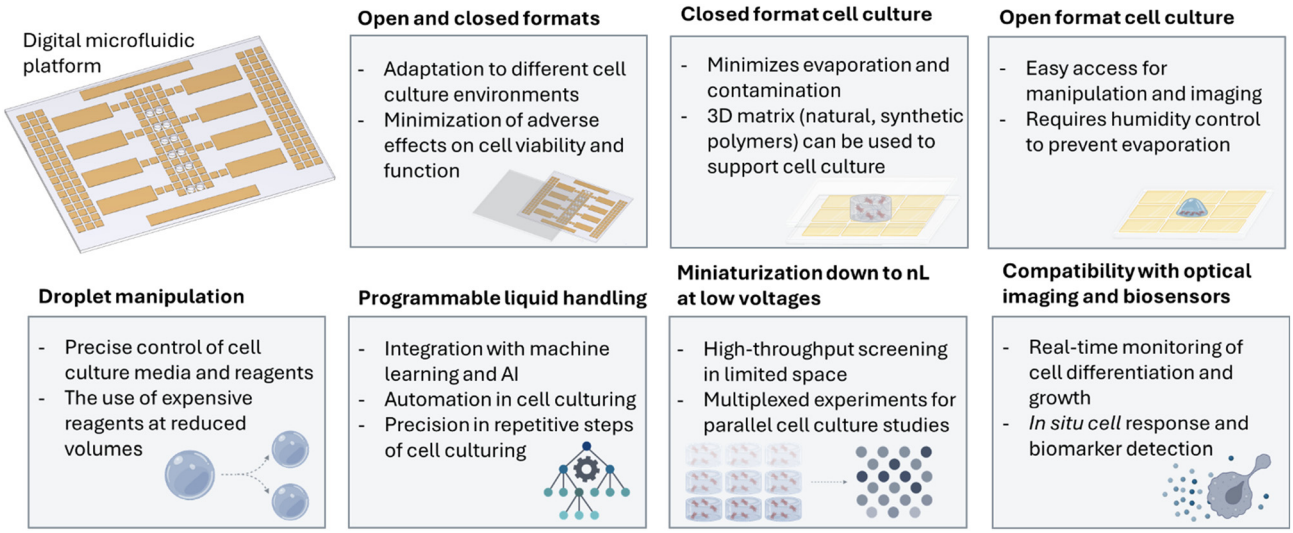


Fig. 1 Basic functions and opportunities offered by the DMF platforms and their associated application areas with specific focus on cell culturing.

populations.[9,10] Lastly, it has just been shown that DMF systems can successfully carry out long-term culture investigations that last up to 60 days, which is the time frame needed for several organ-on-chip models, such as liver cultures and immunology studies.[11]

Apart from the benefits, DMF platforms have a number of drawbacks that should be properly taken into account when performing cell culture studies. First, when the platform is operated, the magnetic field created inside the droplets is a cause for concern. The magnetic flux density in 2–4 μL droplets ranges between −5 ppm and −7 ppm may affect certain sensitive cell types, possibly altering their behaviour, even while no electric field leaks into the droplets.[12] Second, despite the proven capacity of DMF platforms to perform up to 60 days cell culture, the applications of these systems are needed to be expanded to organ-on-chip models, such as liver cultures.[11,13,14] Thirdly, there is a risk of droplet evaporation and leakage, which are particularly problematic when imaging live cultures placed on top of DMF devices. Fourth, a typical restriction is the maximum number of cells that each droplet can support. Cells must usually be pooled for measurements including qPCR, ELISA, and western blotting, which may not be practical because each droplet can only accommodate 500–1000 cells at once. Finally, biofouling of the upper surface of the DMF devices reduces the long-term utility.[15] These challenges, particularly the need for longer-term functionality, compatibility with higher cell loads, and more physiologically relevant conditions, underscore the motivation to integrate organ-on-chip strategies with DMF platforms. Such integration could help address key limitations while leveraging DMF's strengths in automation, miniaturization, and multiplexing, thereby advancing its application in complex, tissue-mimetic studies.

This comprehensive review covers a wide range of topics and emphasizes the benefits and limitations of using DMF systems in cell culture studies. I initially focus on how cell culture experiments are set up and designed inside DMF devices, taking into account how to integrate them with other materials such as hydrogels as cell culture scaffolds, as well as polymers and other materials as dielectric layers. Next, I explore the application of physical and biological stimuli, as well as the utilization of AC and DC modes in DMF. I also discuss integrated analytic tools, cell sorting techniques, and the emerging applications of DMF in organ-on-chip research. Lastly, I discuss current trends and challenges in this rapidly evolving field.

2. Operations

2.1. Minimal requirements in device configuration

In cell culture studies, DMF provides a controlled microenvironment for handling cells, nutrients, and reagents with minimal waste and automation-friendly operation. Designing DMF chips suitable for cell culture requires careful consideration of materials and engineering principles to ensure compatibility with live cells, stable imaging conditions, and efficient droplet actuation. DMF platforms exist in various formats, including printed circuit boards (PCB), thin-film transistor (TFT)-based chips, and active-matrix designs. These architectures differ in their actuation mechanisms, resolution, scalability, and integration potential. PCB-based platforms are cost-effective but often limited in cell culturing applications due to the humidity intolerance of the built-in materials. TFT and active-matrix systems allow for individually addressable electrodes, offering higher spatial resolution and multiplexing, which are advantageous for complex, parallelized cell-based assays and long-term monitoring. These systems typically require more complex fabrication and control schemes and remain less accessible for research labs. In contrast, passive glass-based chips with patterned metal electrodes, the focus of this section, offer an accessible and reproducible platform with sufficient optical transparency and chemical compatibility for biological assays. Their straightforward fabrication and compatibility with standard microscopy setups make them particularly useful for cell culture applications, including droplet-based seeding, stimulation, and *in situ* analysis.

Below, I discuss the structural components of conventional passive glass DMF chips, material choices, construction materials, their role in supporting cell culture, and the challenges associated with biofouling during long-term cell experiments (Fig. 2).

2.1.1. Traditional approaches in the fabrication of dielectric and top layers of a DMF chip for cell culture. Typically, DMF chips are structured on top of glass for cell culture studies, given the stability of glass under humidity and warm temperature conditions when compared to more traditional substrates such as printed circuit boards. Glass also allows for a clear optical path for bright field and fluorescence microscopy. Glass layer houses an array of actuation electrodes often made of a combination of chromium and gold layers, which are coated by a dielectric or insulating layer. Typical materials used as dielectrics include SU-8, PDMS, parylene C, and silicon nitride.[16–18] The final layer is typically hydrophobic coating, which can be Teflon AF, FluoroPel or Cytop next to alternative coatings.[16,18] Such a layer is required to reduce the contact angle of the droplet and helps with reducing the actuation voltage. Actuation electrodes are connected to an often-continuous ground electrode made of indium tin oxide (ITO), which is a transparent and conductive material. It is often deposited on top of the glass substrate *via* dedicated equipment. The connection is made using conductive copper spacers that also define the distance between two plates, therefore the droplet volume to be contained by each electrode. The top plate helps control the evaporation of the droplets and allows for the application of optical monitoring techniques conveniently.

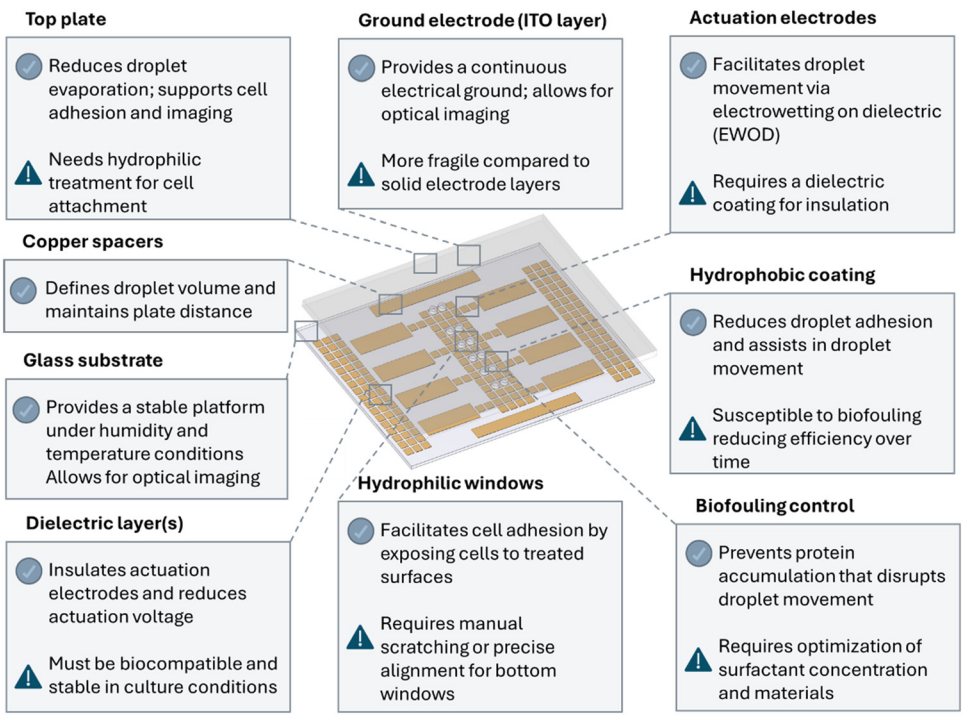


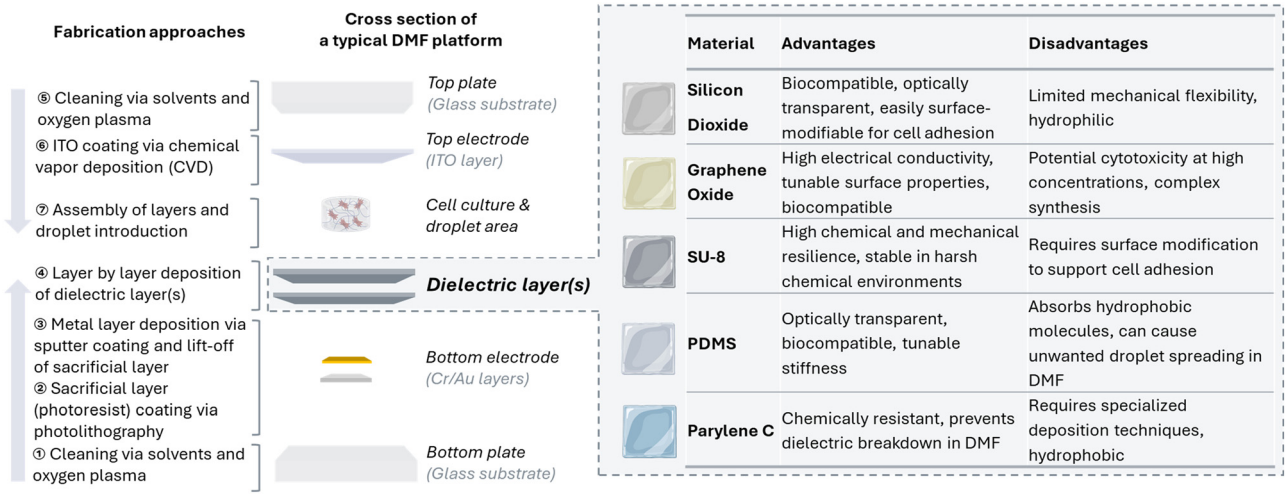
Fig. 2 A summary of minimal requirements in a digital microfluidic device for cell culturing.

Except for certain types of suspension cells (*e.g.*, blood cells, microglia, and macrophages), most mammalian cells necessitate a suitable surface for adhesion and proliferation. Often, cells are cultured on the top plate, which is less complex by design. The culturing is performed by flipping the DMF device in the incubator. By locally removing the ITO and hydrophobic layers, users can easily create hydrophilic windows to settle the cells contained in the droplets. It is possible to coat the surface of the glass within the window with cell adhesion proteins so cells can attach. Opening a window at the bottom plate is also possible yet somewhat more labour intensive due to the alignment of the layers involved. In this way, DMF chips are usually flipped around to enable imaging as the ITO layer is transparent. An alternative of this is to open electrode-free visualization spots at the bottom layer during the electrode design step. As another alternative approach, previous studies have shown that the bottom electrodes can also be fabricated using ITO to enable a fully transparent chip.[19] The configuration is advantageous in terms of droplet visibility and imaging, yet it should be noted that ITO is more fragile when compared to the solid electrode layers.

2.1.2. New approaches in the fabrication of dielectric layer in DMF chips for cell culture. Polymers and other materials have been explored as substrates as dielectric materials in DMF platforms for mammalian cell culturing. The main motivation is to reduce the capacitance generated by the application of electric fields, which, along with magnetic fields and capacitance effects, can influence cell behavior.[44] Despite these materials not being in direct contact with cells due to the hydrophobic coating, they can still be used to create topographies and sections, which may bring them into contact with cells. The common ones are silicon dioxide, graphene oxide, SU-8, and PDMS apart from the commonly used parylene C (Fig. 3). Silicon dioxide has been widely utilized due to its biocompatibility, optical transparency, and ease of surface modification for cell adhesion. Parylene C dominates the use cases in DMF systems because it is a conformal and chemically resistant polymer, which is being used with a wide range of cell types.[45] Next, PDMS has been integrated into DMF systems as it is well-known for good optical clarity, biocompatibility, and adjustable stiffness ranging from 800 kPa to 10 MPa. These features complement the real-time microscopic monitoring compatible DMF systems to track cellular activities. Ahmadi *et al.* automated single-cell droplet-DMF platform by utilizing a PDMS construct to compartmentalize hybridoma and antigen-presenting cells for increasing the throughput screening for monoclonal antibody discovery.[46]

Interestingly, silicon-derived substrates, such as silicon dioxide (SiO_2), have been shown to be non-cytotoxic,[47] yet its use in cell studies remains limited. Graphene oxide is another relatively less used material that has been incorporated into DMF platforms, despite having unique properties such as high surface area, strong electrical connectivity, tuneable wettability, and biocompatibility. Graphene oxide-modified DMF has been used for culturing breast cancer cells and detecting soluble PD-L1 at concentrations as low as 1 pg mL^{-1} *via* the electrochemical sensor arrays for automated, high-sensitivity detection.[48] Finally, the mechanical and chemical resilience of the epoxy-based polymer SU-8 has led to its widespread application in DMF devices despite its relatively low dielectric strength. However, SU-8 by itself does not offer the proper extracellular matrix required to create microsystems for biological uses.

2.1.3. Troubleshooting of the biofouling problem in DMF chips for cell culture. DMF chips allow for droplet operations involving mixing, merging, splitting, combining and transporting when sequence of preprogrammed voltages applied along the electrode array. The working mechanism is



Material	Advantages	Disadvantages
Silicon Dioxide	Biocompatible, optically transparent, easily surface-modifiable for cell adhesion	Limited mechanical flexibility, hydrophilic
Graphene Oxide	High electrical conductivity, tunable surface properties, biocompatible	Potential cytotoxicity at high concentrations, complex synthesis
SU-8	High chemical and mechanical resilience, stable in harsh chemical environments	Requires surface modification to support cell adhesion
PDMS	Optically transparent, biocompatible, tunable stiffness	Absorbs hydrophobic molecules, can cause unwanted droplet spreading in DMF
Parylene C	Chemically resistant, prevents dielectric breakdown in DMF	Requires specialized deposition techniques, hydrophobic

Fig. 3 Cross section of a typical passive glass DMF platform (middle) including the layer composition. General fabrication workflow including example approaches are listed on the left, and a comparison between advantages and disadvantages of the commonly used dielectric materials in DMF platforms for cell culturing are shown on the right.

based on electrowetting on dielectric (EWOD) and has been comprehensively explained previously.[20,21] By versatile droplet actuation operations, gradients, different concentration of cell culture components can be prepared easily. Yet, proteins exist in almost all culture media types. Proteins can immobilize onto the hydrophobic layer *via* hydrophobic reactions[22] and this process is known as biofouling. Biofouling greatly interrupts the droplet actuation by increasing the contact angle of the droplets, thus the electrodes become unusable quickly. To eliminate this effect, the droplets have been either used in core–shell format – where droplets are covered by a thin layer of oil phase, or surfactants such as pluronic have been mixed with the droplets to fight against the contact angle decrease. Recent developments in DMF device fabrication attempted to solve this problem *via* different strategies including the use of different pluronic and tetronic additives,[23] and the application of slippery liquid infused porous surface (SLIPS) surface.[24]

2.1.4. Increasing the throughput in DMF devices. DMF devices are gaining momentum in cell culture research despite longstanding challenges regarding the throughput of the experiments. Several studies have demonstrated innovative solutions that enable DMF devices in both low- and high-throughput settings. In low-throughput systems using regular electrode arrays, the number of addressable electrodes typically remains around 100, often in the range of 16 to 64. For example, Huang *et al.* demonstrated an EWOD-powered digital microfluidic platform with 30 electrodes that enable dynamic single-droplet culture of mammalian embryos in a microfluidic environment—mimicking *in vivo* conditions to significantly improve cleavage to blastocyst rates and yield live births.[25] This is a good example of careful environmental and surface engineering, leading to extension of DMF usability in cell biology. Similarly, Lant *et al.* employed a 19-electrode DMF device to miniaturize and semi-automate cell line optimization for monoclonal antibody production by performing viability, pH, and antibody titter assays in 6–8 μL droplets[26]—suitable for short-term assays but still far from a replacement for Petri dishes or perfusion systems.

In contrast, high-throughput DMF platforms—typically based on active-matrix architectures—feature significantly higher electrode densities, often around 1000 individually addressable units. Azam Shaik *et al.* reported an active-matrix DMF device with 324 electrodes integrated using TFT technology, which enabled infinitely reconfigurable, high-parallelism droplet routing for cell transport and mixing.[27] Building on this flexible droplet control, the same system can be employed to isolate, lyse, and sample single cells, enabling integrated analysis of cellular metabolites directly on-chip. Another notable example is the work by Zhai *et al.*, who incorporated on-chip 3D microstructures forms semi-closed microwells that achieve ~20% single-cell capture across 900 electrodes while using low-evaporation oil and surfactant to reduce actuation voltage to 36 V—enabling gentle, long-term culture and reliable on-chip drug sensitivity testing comparable to 96-well plates.[28] This is an important step to parallelize single-cell capture and long-term culture with improved chip durability, cell viability, and assay reliability while slashing reagent consumption.

Together, these studies underscore the evolving capabilities of DMF for cell culture: regular low-electrode-count devices are being refined for specialized tasks such as single-cell assays or differentiation protocols, while high-electrode-count active-matrix systems push toward scalable, high-content biological screening. Despite remaining limitations, especially for long-term or high-density cultures, the field is transitioning rapidly due to innovations in materials, device integration, and environmental control. As such, the number and configuration of electrodes—ranging from a few dozen in low-throughput to thousands in active-matrix designs—play a central role in shaping the operational scope and biological applicability of DMF-based culture platforms.

2.2. Cell culturing

Adherent cells are the cornerstone of organ-on-chip systems because their interactions with the extracellular matrix (ECM) and neighbouring cells drive the very functions these platforms aim to replicate—barrier integrity, mechanotransduction, and multicellular signalling. By anchoring onto ECM-coated substrates, cells polarize, form tight junctions, and generate the traction forces necessary for realistic tissue mechanics. Maintaining these adhesive phenotypes over time is therefore essential because loss of adhesion not only impairs viability but also disrupts the spatial patterning and function of engineered microtissues. For this reason, I focus on the adherent cell cultures in DMF chips in this review.

DMF chips excel in single-droplet control with each droplet containing from 100 nL to a few μL that can be independently actuated, merged, split, or dispensed. As a result, DMF chips can minimize reagent consumption and virtually eliminate cross-contamination. Yet, translating this precision into a stable platform for long-term adherent cultures requires balancing fluidic components with surface integrity. Biologically, adherent cells demand constant humidity, nutrient replenishment, and mechanical support. Covering droplets with an inert oil overlay or housing the chip in a humidified enclosure can prevent evaporation, while scheduled droplet exchanges supply fresh media. But these techniques alone cannot safeguard the long-term performance of DMF chips.

At the material level, repeated electrowetting cycles and protein adsorption progressively degrade the hydrophobic dielectric layers. Proteins and lipids bind to fluoropolymer coatings, increasing contact-angle hysteresis and necessitating ever-higher voltages for droplet movement. To counteract this, surface chemistry as well as dielectric layers can be altered. Structurally, multilayer dielectrics—such as

the reinforced two-layer stack demonstrated in the 60-day DMF chip[11]—enhance breakdown strength and reduce leakage currents, enabling low-voltage operation (<50 V) compatible with sensitive cell types such as macrophages. Beyond coatings, incorporating inline pH, oxygen, and metabolite sensors directly into the DMF footprint not only eliminates complex tubing but also provides real-time feedback to dynamically adjust actuation protocols. Finally, pairing these advances with modular organoid chambers—pre-patterned with collagen or fibrin gels—allows three-dimensional tissue constructs to be cultured under DMF control, bridging the gap between droplet manipulation and true organ-level function.

By weaving these material innovations, fluidic architectures, and sensing modalities into the DMF chips, the long-term, high-density adherent cultures required for organ-on-chip applications can be achieved—ultimately creating a unified system that leverages the automation and multiplexing of DMF for studying complex biological processes.

2.2.1. DMF chips in 2D and 3D adherent cell cultures. DMF chips allow for both 2D and 3D adherent cell culture formats. To enable cell culturing in any format, the hydrophobic layer used on the bottom or top surface needs to be modified either by removing it or by coating it with proteins such as arginylglycylaspartic acid (RGD) peptide, ECM proteins, fibronectin or laminin; or hydrogels such as agarose or collagen.[29] This can be done typically by incubating the chip surface with cell culture media or phosphate buffered saline (PBS) solution overnight.

2D culturing format. The initial cell lines selected were commercially available immortal cell lines, which were later followed by primary cell lines. The first complete cell culture handling was achieved in 2D format in 2010 by Barbulovic-Nad *et al.*,[30] where ECM proteins were deposited at bottom plate on the hydrophobic surface of DMF for a full cycle of cell seeding, culture medium refreshing, cell detachment and reseeding of CHO-K1 and HeLa cells. Although done successfully, irregular attachment and detachment of ECM proteins led to a low reproducibility rate. Following this, more reproducible cultures were ensured by lifting off geometrically designed patterns on the top plate, leaving the bare glass accessible for the droplets as discussed earlier. In this way, consistent monoculture of each MDCK and HeLa cell lines was demonstrated by Eydelnant *et al.*[31] This was followed by 2D co-culture by Srigunapalan *et al.* where three types of primary aortic cells were co-cultured for 7 days and also analysed by fluorescent immunostaining.[32] 2D adherent cell culture format has continued to grow after these first demonstrations. Recent co-culture applications involve drug screening on the co-culture of MCF-10A and MDA-MB-231 breast cancer cell lines using cisplatin and epirubicin.[33] Although not co-culture, cell differentiation has been also explored in DMF chips. Yu *et al.* differentiated SH-SY-5Y cells into neurons and explored time- and concentration-dependent dopamine homeostasis.[34] Zhai *et al.* introduced a jetting bar functioning as a drug dispensing unit which can dispense satellite droplets of two different drugs taken up by MDA-MB-231 and MCF-10A cells.[35] Here, precise control over droplet ejection position and volume was achieved using a narrow electrode, known as a jetting bar, which concentrates the high-voltage AC actuation to a localized area. When the electric field intensity rapidly exceeds the contact angle saturation threshold, the excess energy causes the emission of satellite droplets. These picolitre-sized droplets are dispensed onto the jetting bar and subsequently collected by a larger droplet passing over it. By repeating this process, droplet volumes ranging from 5 picolitres to 20 nanolitres can be generated.

3D cell culturing format. 3D cell culturing format found wide use in DMF chips which paved the way to more physiologically relevant cell microenvironment. The first 3D format has been applied by Fiddes *et al.*, where agarose hydrogel discs were utilized to create 3D microenvironment for NIH-3 T3 cells. The system allowed the exchange of culture medium both actively and passively as the hydrogel discs while it did not change their location during the droplet actuation thanks to the use of the hydrophobic windows.[36] Bender *et al.* applied 3D format structured in a hanging droplet, where fibroblasts spheroids were grown in collagen matrix.[37] Another inspiring use of 3D hydrogel scaffold has been proposed for cell invasion assay, where MDA-MB-231 cells were sent through a collagen type I hydrogel matrix to invade the free space by the help of gravity.[38] Through changing the electrode design, it is also possible to create 3D patterns made of hydrogels in DMF. Several examples of such electrode format include a ring-shaped alginate gel for testing different concentrations of DMSO for cell viability.[35] Another exciting application is the 3D patterning of polyethylene glycol diacrylate (PEG-DA), Matrigel, polyacrylamide and gelatin methacryloyl (Gel-MA) with heterogenous architecture representing topography changes for 3D cell culture. The patterning was achieved using dielectrophoresis and electrowetting. These constructs have been applied to culture NIH-3 T3 fibroblasts for 48 hours.[39] In another work, a co-culture of HepG2 and NIH-3 T3 cells embedded in collagen hydrogel matrices has been shown to form organoids, on which different concentrations of acetaminophen have been tested.[40]

2.2.2. Setting up the scene for adherent cultures: cell culture in DMF-embedded scaffolds. Building organ-on-chip constructs using DMF chips has been explored thanks to their precise droplet manipulation, automated control over microenvironments, and the ability to integrate multiple cell types in a miniaturized, scalable platform. A functional and versatile bridge for connecting organ-on-chip constructs and DMF is hydrogels. For this reason, integration of many different hydrogel types has been explored. Among all these types, collagen, Gel-MA, PEG-DA, alginate, agarose and Matrigel remain the most used ones in DMF platforms (Table 2). Either chemical or photo crosslinking hydrogels are mainly preferred for the ease of patterning. The optical

Table 2 Comparison between advantages and disadvantages of the commonly used hydrogels as scaffolds in DMF platforms for cell culturing

Material	Advantages	Disadvantages
Collagen	Excellent biocompatibility, promotes cell adhesion and proliferation, supports 3D cell culture, integrates well with DMF for spheroid-based invasion assays	Poor mechanical strength, degrades over time, high batch-to-batch variability, difficult to precisely manipulate in DMF
Gel-MA	Tunable mechanical properties, good biocompatibility, photocrosslinkable for patterning, good for rapid cell trapping and then culturing in DMF	Requires UV exposure for polymerization, potential cytotoxicity from photoinitiators
PEG-DA	Non-toxic, tunable stiffness, allows precise on-chip photopolymerization, supports cell adhesion studies in DMF platforms	Requires surface functionalization, polymerization parameters must be optimized for DMF platforms
Alginate	Biocompatible, easy to gel using calcium ions, enables 3D encapsulation, can be used in DMF for toxicity assays	Poor cell adhesion, requires RGD modification for better bioactivity, viscosity can hinder precise DMF droplet manipulation
Agarose	Thermoresponsive gelation, inert and stable structure, may allow for solvent exchange in DMF	Limited cell adhesion, not biodegradable, lacks tunability, challenging to integrate with active droplet actuation in DMF
Matrigel	Rich in ECM proteins, supports organoid and spheroid formation, provides a highly bioactive environment compatible with DMF	High batch-to-batch variability, expensive, undefined composition, handling in DMF can be inconsistent due to its temperature sensitivity

transparency of the top and bottom substrates (glass, ITO layers) makes it easy to apply photo crosslinking. Chemical crosslinking can be controlled *via* temperature (on ice or in incubator incubations of hydrogels) while maintaining the electrode activation. This does not create application problems as the crosslinking times are generally fast. Fiddes *et al.* cultivated NIH 3 T3 cells within cylindrical agarose discs, measuring 140 μm in height, enabling successful solvent exchange. Furthermore, they functionalized these hydrogel discs with proteins to serve as enzymatic microreactors, exemplified by the action of alkaline phosphatase on fluorescein diphosphate.[36] In another study, alginate has been used to 3D culture MCF-7 cells, where the cytotoxicity of different DMSO concentrations was tested.[41] Chiang *et al.* integrated PEG-DA within DMF to enable on-chip photopolymerization of hydrogel microstructures for cell culture. Using UV exposure for selective patterning, PEG-DA droplets were polymerized and used for NIH-3 T3 fibroblast adhesion with tuneable mechanical properties of the adhesion surface.[42] Using sol–gel collagen discs, spheroid-based invasion assays have been developed in DMF, which allowed for separate droplets of HT-29 human colorectal adenocarcinoma cells and BJ human fibroblasts brought up together to stimulate a cell invasion model.[37] Lastly, Gel-MA-incorporated DMF chip enabled 3D cell culture by utilizing dielectrophoresis to automatically trap and pattern HEK 293 cells within hydrogel structures on planar electrode traps, demonstrating fast cell patterning (in order of 10 to 100 s of seconds), and viability up to 78% after 4 days.[43]

DMF chips leverage the precise, automated handling of discrete droplets to integrate diverse photo- and chemically crosslinkable hydrogels—such as collagen, Gel-MA, PEG-DA, alginate, agarose, and Matrigel—into miniaturized organ-on-chip scaffolds, enabling rapid patterning, tuneable mechanics, and applications ranging from enzymatic microreactors and toxicity assays to 3D spheroid invasion models and dielectrophoretic cell trapping with sustained viability. By embedding the hydrogel constructs, DMF chips gain not only structural fidelity but also localized control over electrical and chemical cues—setting the stage for tailored actuation strategies that respect the delicate balance of cell health and scaffold stability.

2.2.3. Application of AC and DC modes for droplet actuation. Voltage can be applied in DMF devices either in alternating current (AC) or direct current (DC) actuation mode, which can significantly influence droplet velocity, reliability, stability and device use time. DMF still faces critical challenges in cell-based experiments, particularly regarding the choice of actuation mode. AC actuation is more common than DC actuation because it does not stimulate electrolysis, thereby maintaining a stable microenvironment for cells. It enhances droplet motion through EWOD mechanisms without excessive heating. Furthermore, AC fields can be tuned to manipulate cells using dielectrophoresis (DEP), which enables selective cell sorting based on intrinsic electrical properties. For example, neural progenitor cells exhibit enhanced differentiation when exposed to specific AC field parameters for tissue engineering applications.[49,50] AC actuation can be advantageous for long-term cell culture studies, as it prevents the buildup of electrochemical byproducts that could otherwise compromise cellular health. The frequency and strength of the applied electric field changes based on the purpose. Most commercial power supplies that are connected to DMF devices use AC format.

In contrast, DC actuation can lead to electrolysis, bubble formation, and pH shifts, which may compromise cell viability. However, DC-based DMF has been mostly utilized for applications, where high droplet transport efficiency, such as electrochemical sensing and direct current-induced electroporation for gene delivery, are needed. Tian *et al.* has reported that controlled DC pulses can selectively lyse cells or permeabilize membranes, which is particularly useful for drug screening and genetic modification applications.[51] To mitigate adverse effects, hybrid AC/DC actuation has been

applied.[52] Especially, Bojarevics & Pericleous *et al.* showed that droplet oscillations in high-gradient static magnetic fields induce deformation and oscillatory motion in conductive droplets. These results provide a non-contact actuation technique for lab-on-a-chip applications and aid in the development of magnetically controlled droplet manipulation in microfluidics.[52–54] Apart from cell culturing, DC actuation has been mostly applied to electrophoretic manipulations, where charged biomolecules such as DNA and proteins are efficiently transported across microfluidic channels. This makes DC-based DMF particularly valuable in molecular biology workflows, including PCR-based diagnostics and nucleic acid hybridization assays as the end point analysis after cell culture.[55]

Magnetic field generation is a direct consequence of electric field application in DMF systems, and although minimal, cell behaviour can be influenced by the magnetic field presence.[44] The effects of electric and magnetic fields on cells vary based on actuation mode. AC fields, especially in the radiofrequency range, have been shown to promote cellular responses such as differentiation and proliferation. For instance, alternating fields have been employed to guide neuronal differentiation in microfluidic devices, enhancing their potential for neurobiology research.[50] On the other hand, DC fields are often used in microfluidic setups to induce electrophoretic movement of charged biomolecules and control cellular orientation in culture systems, as discussed earlier. Magnetic actuation, often coupled with AC or DC formats, allows for label-free cell manipulation by integrating magnetic nanoparticles into the droplets. This technique has proven useful for isolating circulating tumour cells from whole blood samples.[56] Magnetic actuation has also been explored in the context of tissue engineering, where magnetically labelled stem cells can be remotely guided to assemble into functional tissue structures.[57] AC actuation in DMF is preferred for its gentle, electrolysis-free droplet control and tuneable dielectrophoretic cell manipulation—ideal for long-term cultures—while DC offers rapid, high-efficiency transport and membrane-permeabilizing pulses for applications like electroporation and sensing, with hybrid AC/DC and incidental magnetic fields further expanding programmable stimulation modalities. By integrating programmable AC/DC-driven electrical inputs with finely orchestrated droplet manipulations, DMF-embedded platforms achieve the multifactorial control necessary to drive physiologically relevant cell behaviour *in vitro* and create a wide range of possibilities to apply physical and biochemical stimuli which cannot be achieved otherwise.

2.3. Physical and biochemical stimuli in DMF chips

Modulating or monitoring cell behaviour requires precise control of the cellular microenvironment provided by the integration of physiologically relevant cues in DMF platforms. While organ-on-chip systems paved the way to advanced functionalities over the last decades, the precision of *in vitro* research can still be improved by adjusting mechanical pressure, electrical signal, chemical gradient, and fluid shear

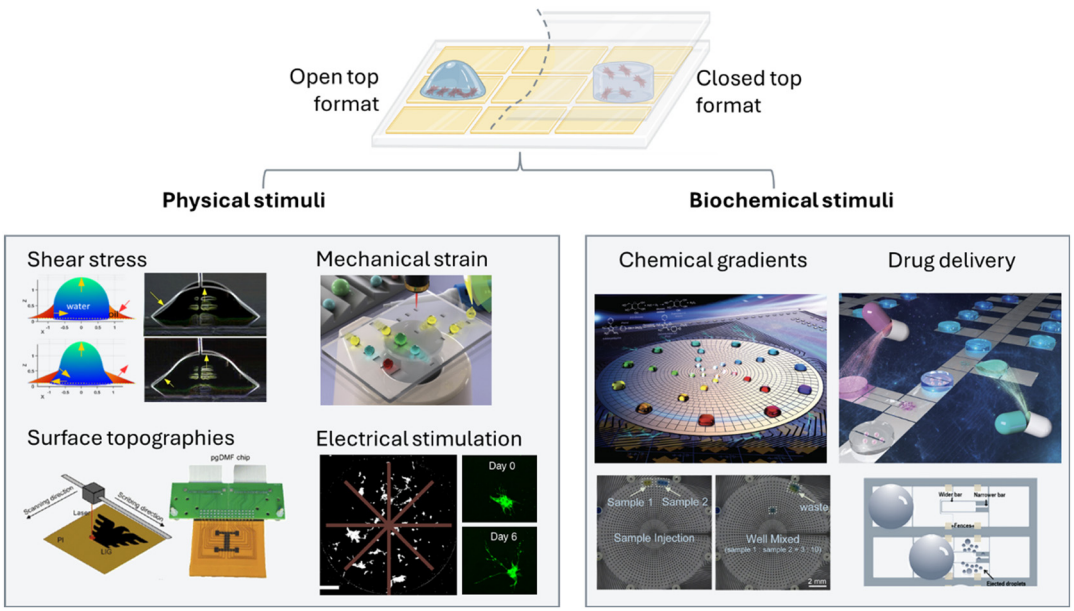


Fig. 4 Examples of applied physical and biochemical stimuli in DMF platforms in open and closed formats. The shear stress image has been adapted from ref. 58 with permission from ACS Publications, copyright 2017; the mechanical strain image has been adapted from ref. 60 with permission from RSC Publications, copyright 2019; the surface topographies image has been adapted from ref. 68 with permission from RSC Publications, copyright 2024; the electrical stimulation image has been adapted from ref. 34 with open access permission from Nature Publishing Group, copyright 2019; the chemical gradients image has been adapted from ref. 72 with permission from RSC Publications, copyright 2024; the drug delivery image has been adapted from ref. 35 with permission from RSC Publications, copyright 2021.

stress with miniaturized scales. Utilizing the adjustable stimuli, DMF-based systems bridge the gap between traditional organ-on-chip approaches and the technical side of the stimuli application, enabling sophisticated applications in cell culture (Fig. 4).

2.3.1. Physical stimuli. While fluid flow creates **shear stress**, crucial for many cell types, implementing controlled flow in DMF is rather challenging due to the individual droplet manipulation nature of the platform. However, methods like oscillating droplets or creating "virtual channels" *via* sequential droplet actuation can mimic shear stress. For example, Bansal *et al.* investigated the oscillatory dynamics of EWOD-actuated compound droplets with an aqueous core and oil shell, revealing that reduced shell volume minimizes damping and enhances both axisymmetric and nonaxisymmetric oscillations. The optimized frequency regimes enabled efficient mixing and biofluid manipulation, which can be potentially used for rapid assays and fluidic photonics while mitigating biofouling and evaporation in cell experiments.[58] The theory of shear stress effects in a droplet has been discussed by Hu *et al.* Here, a triangular geometrical model enhanced pressure differentials for controllable sub-droplet formation in confined geometries thanks to the distortion of droplets.[59]

Cells experience **mechanical strain** due to movement and external forces. Mechanical strain application is typically characterized by consecutive stretching and relaxation movements of the scaffold where adherent cells attach. The examples of the application of mechanical strain are not common in DMF devices given the same reason as the shear stress. Yet, Qi *et al.* demonstrated that droplet stretching can be applied in such platforms. This was made possible by a laser-irradiated superhydrophobic surface with patterned gradient wettability, enabling long-distance aqueous droplet transport, mixing, and selective manipulation under in-plane cyclic vibration.[60] In the future, integrating flexible membranes or actuators within DMF chips may allow for the application of controlled stretching or compression to mimic physiological conditions, which can improve health and disease modelling in heart, vessel, muscle, lung tissues in regenerative medicine.[61–64]

Electrical stimulation becomes a crucial factor in regulating the activity and functional responses of excitable tissues, such as neurons, heart and muscle cells. DMF platforms innately facilitate a versatile approach for integrating electrodes and precisely applying and precisely controlling electric fields, facilitating real-time observation of cellular dynamics and signal transmission. This can be achieved by activating metal electrodes. Yu *et al.* demonstrated a DMF platform integrating voltametric dopamine sensors (LOD: 30 nM) with neuron culture sites enables high-throughput, in-line analysis of dopamine uptake (~32 fmol/10 min per microwell with ~200 SH-SY5Y cells), addressing automation and sensitivity limitations of traditional methods and advancing neurodegenerative disease research in DMF-based screening.[34] Next, Pavesi *et al.* utilized a PDMS based micro-scale cell stimulator that integrates mechanical stretching, uniform electrical field application, and controlled biochemical delivery within a single platform, enabling precise and reproducible human bone marrow mesenchymal stem cell differentiation studies.[65] Although this platform was not a DMF device, it still presents a well applicable concept in DMF platforms. Furthermore, the ability to combine electrical stimulation with other microenvironmental cues, such as biochemical gradients and mechanical forces, makes DMF platforms particularly attractive for studying complex cellular behaviours. A comprehensive review by Pramotton *et al.* highlighted the potential of micro electrophysiological devices in creating physiologically relevant *in vitro* models for neurodegenerative diseases.[66]

Cells respond to **topographical cues** on the substrate that they are cultured. Microfabrication techniques can create defined topographies within DMF chips, enabling investigation of cell adhesion and differentiation. The topographies involve periodic or random patterns of shapes with certain height, typically up to tens to hundreds of microns based on the application. Research on cell behaviour on patterned surfaces within DMF devices is an active area, yet there are multiple cell-free explorations on pattern formation directly on top of the electrodes. For instance, Zhang *et al.* recently presented a DMF chip that integrates surface wettability gradients with surface acoustic waves (SAWs) to achieve precise multi-droplet manipulation, where photolithographically patterned octagonal wetting gradients on $LiNbO_3$ wafers, combined with interdigital transducers, enable selective droplet actuation, sequential reactions, and micrometre-level positional accuracy, advancing high-precision droplet control in DMF.[67] Instead of photolithography, laser-induced writing can also be used in topography generation in DMF devices. Liu *et al.* used laser-induced graphene-based DMF platform, which can be fabricated within 10 minutes under ambient conditions, to enable planar- and vertical-electrode addressing for patterned structures and electrodes.[68] Surface patterning is also employed in the form of hydrophobic or hydrophilic coatings in DMF platforms. As a recent example among many others, Kašpar *et al.* reported the use of micro-patterned surfaces with alternating hydrophilic and hydrophobic rectangular areas to confine water droplets down to attoliter volumes. The rectangular areas are created as previously discussed in section 2.1. This combined experimental and simulation approach facilitates the purposeful design of arrays with surface-addressable hydrophobicity which finds multiple uses in microscale cell culturing.[69] Apart from this, inkjet printers have also been introduced for DMF device construction, especially for cost-effective, high-performance diagnostic assays.[70,71]

DMF chips have the potential to generale key physical and biochemical microenvironmental cues—shear stress *via* oscillating droplets or virtual channels, mechanical strain through droplet stretching on superhydrophobic gradients,

precise electrical stimulation with integrated electrodes, and defined topographical guidance using photolithographic or laser-induced surface patterning—within a single, miniaturized device.

2.3.2. Biochemical stimuli. Controlled **chemical gradients**, essential for studying cellular manipulation such as gene editing and drug responses, can be precisely generated using DMF, which can generate stable gradients through droplet mixing, splitting, merging, and thus serial dilution. Chemical gradient generation in DMF devices is facilitated by the low Reynolds number regime in which these devices operate. At the microscale, fluid flow is dominated by viscous forces rather than inertial forces, resulting in highly predictable, laminar flow with minimal mixing due to turbulence. In this regime ($Re < 1$), droplets remain well-contained and mixing occurs primarily through diffusion and controlled droplet merging rather than chaotic flow. This allows DMF systems to perform precise, stepwise dilution by merging droplets of different concentrations and volumes, enabling the construction of stable and reproducible chemical gradients. Because flow is deterministic at low Reynolds numbers, the spatial and temporal characteristics of the gradients can be tightly controlled, making DMF a powerful tool for applications such as cell signalling studies, chemotaxis assays, or drug screening. A very recent example of an active-matrix DMF (AM-DMF) device with a polar coordinate electrode arrangement enabled high-resolution concentration gradient generation with a 19-fold improvement over conventional rectangular layouts by utilizing 33 electrode sizes to produce droplets of varying volumes with <3% variation, demonstrating precise quantification of biochemical and glucose concentrations with correlation coefficients above 0.99. Although this study did not involve any cell culturing work, it stands out as a great example of gradient formation capacity of DMF platforms which can also be used for cell culturing.[72] In another example, Quach *et al.* presented a DMF platform that automates lentiviral generation, packaging, and transduction, enabling precise dilution control and efficient cell culture handling while achieving viral titters of 10^6–10^7 and high transduction efficiency, demonstrating its applicability for gene editing, including oestrogen receptor knockout in breast cancer research.[73] Additionally, the work of Yang *et al.* is an example of sample dilution and high-throughput analysis, as it identified an average of 2258 protein groups per HeLa cell within 15 minutes, and demonstrated DMF's potential for studying drug resistance mechanisms through comparative tumour cell line analyses.[74]

Localized drug delivery in cell cultures is another prominent application of DMF technology. This method allows for targeted delivery of drugs to specific cell populations or regions within a microfluidic chip. Zhai *et al.* accomplished parallel screening of three drugs within a 4 × 4 cm^2 DMF chip. The system automated droplet manipulation for precise drug delivery and cell culture, optimizing resource efficiency while maintaining assay accuracy. In this platform, an MDA-MB-231 breast cancer xenograft model and liver cancer patient samples were used to validate DMF's capability to identify effective treatments, with on-chip drug responses correlating with tumour genetic profiles from exome sequencing.[7] Another work from the same research group followed in the same year that they presented a DMF platform with an innovative control structure and chip design for automated on-chip drug dispensing, enabling concentration gradients spanning three to four orders of magnitude for single and combinatorial drug screening. The system utilized high-voltage pulse actuation to eject precise drug volumes from a specialized dispenser electrode, which are subsequently merged with cell suspension droplets under low-voltage sine wave actuation. Validation with cisplatin and epirubicin on MDA-MB-231 breast cancer cells and MCF-10A normal breast cells demonstrated results consistent with conventional 96-well plate assays.[35]

Controlled chemical gradients in DMF devices leverage low-Reynolds-number laminar flow and precise droplet operations—mixing, splitting, and merging—to create stable, reproducible stepwise dilutions for applications ranging from chemotaxis assays to high-throughput drug screening and localized drug delivery.

2.4. Cell sorting and bioprinting

Cell sorting and bioprinting have emerged as common applications of DMF chips as they require fast processing, the individual control of small droplet volumes containing biological components.

2.4.1. Cell sorting. Cell sorting is a fundamental process in biomedical research and clinical diagnostics, enabling the isolation of specific cell populations from heterogeneous mixtures. Traditional methods, such as fluorescence-activated cell sorting (FACS), are often bulky and require extensive sample preparation. In contrast, DMF platforms offer a miniaturized and efficient alternative. One notable advancement in this area is the development of laser-based switching mechanisms for high-speed droplet sorting. Wunenburger *et al.* demonstrated the use of thermocapillary stresses induced by laser irradiation to manipulate flowing droplets, achieving switching efficiencies of 100% for droplet velocities up to certain thresholds. This method facilitated the sorting of droplets without the need for physical microfabricated structures, thereby simplifying the chip design and enhancing flexibility.[75] In this approach, cell sorting part did not take place, but the concept stands as well applicable to cell sorting. As another innovative approach, diamagnetic manipulation within DMF platforms exploited the intrinsic magnetic properties of cells and droplets, allowing for efficient sorting without external labels.[76] The importance of this work lies in the fact that label-free technique reduces potential alterations to cell physiology. The incorporation of deep learning in cell sorting can easily be implemented in DMF platforms. As a novel demonstration, an AM-DMF platform integrated deep

learning-based image recognition (YOLOv8) and Safe Interval Path Planning for precise, collision-free droplet manipulation, achieving 98.5% sorting precision, 96.49% purity, and 80% recovery over three cycles for HeLa cells and polystyrene beads, with successful application to various cell types and on-chip lysis for downstream bioanalysis.[77] With this, the potential of DMF for high throughput analysis reaches a significant peak. For example, hardware capacity has been significantly increased with another AM-DMF platform with 26 368 independently addressable electrodes facilitated high-resolution digital droplet generation (~100 μm diameter, 500 pL volume) and precise single-cell manipulation, achieving over 98% success in droplet formation, a pristine single-cell generation rate of 29%, stable droplet movement for over 1 hour, and on-chip cell culturing with 12.5% proliferation after 20 hours.[78]

2.4.2. Bioprinting. Bioprinting aims to fabricate complex tissue constructs by precisely positioning cells and biomaterials in three-dimensional space. Integrating DMF technology into bioprinting platforms offers several advantages, including enhanced control over cell placement and the ability to create heterogeneous tissue structures. DMF can be used as a tool for bioprinting. A preliminary example of this has been shown by George *et al.*, where EWOD-based DMF enabled bioprinting of uniform alginate hydrogel structures for 3D cell culture and chemical screening, facilitating precise liquid handling without physical barriers or hydrophilic patterning, and allowing temporal analysis of chemical effects on hydrogel-encapsulated cells.[79] A cell-free demonstration of controlling droplet detachment dynamics and vertical gradient formation can be exemplified for hydrogel-based microtissue construction. Hong *et al.*, modulated surface energy through square-pulse electrowetting actuation, where the threshold voltage for droplet detachment in high-viscosity silicone oil was reduced by ~70%, allowing controlled deposition of HUVEC-collagen (4×10^4 cells per mL) bioinks.[80] Additionally, AC and DC electrowetting facilitate vertical gradient formation by precisely layering bioinks with varying compositions, supporting cell differentiation and tissue patterning.

DMF's ability to transport, merge, and mix droplets on patterned electrodes further enhances its application in structured hydrogel-based bioprinting and advanced cell-based assays. Microfluidics-enhanced extrusion bioprinting represents a significant leap forward in this domain. By incorporating microfluidic channels into the printhead design, finer control over bioink deposition can be achieved, leading to improved resolution and structural integrity of bioprinted tissues. In this way, hydrogel-based microtissues can be bioprinted to construct complex tissue architectures. These microtissue constructs created in droplets serve as building blocks for tissue engineering applications. For example, they can encapsulate cells, proteins, or other bioactive molecules, facilitating the creation of functional microtissues with controlled microenvironments. However, challenges such as increased shear stress and potential clogging of microchannels necessitate careful optimization of the system.[81] As a potential solution, bioprinting in DMF can be supported by digital light processing (DLP) bioprinting. This approach utilizes a digital micromirror device to project light patterns onto a photopolymerizable bioink, enabling rapid and precise layer-by-layer fabrication of tissue constructs. The integration of microfluidic chips allows for swift switching between different bioinks, facilitating the creation of multi-material and functionally graded tissues.[82] Following this trend, microfluidic-assisted bioprinting techniques have been employed to fabricate vascularized tissues by precisely controlling the deposition of endothelial cells and supporting matrices. These advancements hold significant potential for tissue engineering and regenerative medicine applications.[83]

DMF chips enable rapid, label-free, and high-precision cell sorting and bioprinting—leveraging techniques such as thermocapillary switching, diamagnetic manipulation, and AI-guided droplet control—to achieve high purity, recovery, and spatial patterning of diverse cell types. Building on this droplet-level precision, the same platforms can seamlessly interface with analytical workflows for on-chip sampling and metabolite profiling, enabling integrated analysis of cellular secretions and intracellular metabolites.

2.5. Analysis of cells and metabolites

DMF enables precise manipulation of nanolitre-sized droplets, facilitating high-throughput and automated analyses, which are particularly interesting for complex biochemical assays, impedance measurements, toxicity assays, immunoassays, integration of measurement equipment, and the incorporation of sensors within DMF platforms (Table 3). The pioneering work to introduce cell culture in DMF platforms is the paper from Barbulovic-Nad *et al.* who designed a DMF chip for complete mammalian cell culture processes, including cell seeding, growth, detachment, and re-seeding.[30] After this, streamlining cell-based assays have started in DMF chips, bringing reduced contamination risks and increased capacity to automate the cell culturing process. This was brought one step further when Hu *et al.* introduced a high-resolution AM-DMF system designed for parallel single-cell manipulation.[78] The platform was based on a parallel plate configuration and was made possible by large-scale flat-panel display manufacturing method. It successfully generated and transported droplets containing individual HEK 293 cells. Droplets in these platforms can be operated in air and in oil modes, and this stands out as an important caveat when designing experiments and cell culturing durations in such devices. Table 3 summarizes all the techniques being discussed in the following paragraphs.

2.5.1. Metabolite detection and assays. As one of the most prominent applications, the ability to manipulate picolitre to microliter volumes enables the isolation of single cells and **nucleic acid analysis**. By integrating an advanced isolation

Table 3 The summary of the applications, advantages, and disadvantages of DMF in cell and metabolite analysis with corresponding references

Category	Application	Advantages	Disadvantages	References
Cell culture	Complete mammalian cell culture, including seeding, growth, detachment, and re-seeding	Reduces contamination risks, enables automation	Limited scalability, requires optimization for different cell types	25, 74
Single-cell manipulation	High-resolution AM-DMF system for parallel single-cell handling	Precise droplet control, compatible with large-scale manufacturing	Requires rather complex fabrication, potential cell viability concerns	74, 80
Single-cell isolation and nucleic acid analysis	On-chip single-cell isolation and DNA amplification	High genome coverage, reproducible results	Sensitive to contamination	80–82
Single-cell transcriptomics	Automated nucleotide capture and sequencing	High detection sensitivity, cost-effective compared to tube-based methods	Sample loss during processing, requires careful optimization	81, 82
Simultaneous nuclear and transcriptomic analysis	Combined nuclear DNA and RNA sequencing	Reduces amplification bias, enhances detection sensitivity	Complex workflow, requires specialized reagents	82
Metabolite detection	Enzyme activity analysis from human liver microsomes	Requires minimal sample volume, enables parallel analysis	Limited to specific enzyme assays, requires specialized microsomal preparations	83–85
Ribozymatic cleavage monitoring	Fluorescence-based real-time monitoring	Eliminates need for gel electrophoresis, enables rapid processing	Fluorescence detection may require expensive optics	84, 85
ELISA testing	Open-source DMF platform for immunoassays	Low-cost, customizable, and automation-friendly	Surface functionalization may degrade over time	85, 86, 91
Immunoassays	Electrochemical and magnetic-based immunoassays	High sensitivity, real-time detection	Electrode modification complexity, potential signal interference	86–91
Impedance-based analysis	Real-time droplet and cell monitoring	Non-invasive, high sensitivity	Susceptible to signal noise, requires careful calibration	92–95
Mass spectrometry integration	On-the-fly MS analysis of droplets	Real-time analysis, minimal sample consumption	Requires precise alignment and integration with MS system	96–98
SERS-based metabolite analysis	DMF-SERS integration for Raman analysis	High classification accuracy, minimal sample preparation	Requires specialized substrates, potential memory effects	99–101
NMR integration	Real-time metabolomic analysis in DMF	Non-destructive analysis, high specificity	Requires specialized microcoils, complex integration	102, 103
FET-based biosensors	Electrical detection of biomolecules in DMF droplets	Label-free detection, integrates with existing electronics	Requires stable electrical conditions, sensitivity to environmental noise	104
Surface acoustic waves	Contactless droplet manipulation and heating	Precise droplet control, rapid temperature regulation	Requires piezoelectric materials, potential heating limitations	62, 105

strategy with on-chip multiple displacement amplification, Xu *et al.*'s DMF platform efficiently performed single-cell isolation and lysis, achieving consistent multiple displacement amplification results (CV = 0.15) and comparable genome coverage to bulk methods.[84] Later Zhang *et al.* developed a cell-in-library-out single-cell transcriptome sequencing, which could automate nucleotide capture, efficient purification with minimal loss, and magnetic bead-based elution, leading to highly reproducible reactions at the hydrophobic interface (R = 0.98) with a 1.4-fold improvement in detection sensitivity while reducing preparation costs by 90% compared to conventional tube-based methods.[85] Further advancing the technology, Xu *et al.* could process nuclear genomic and transcriptomic content from the same cell simultaneously as an exciting expansion of DMF technology.[86] Being considered as the state-of-the-art method, DMF DNA/RNA-seq approach integrated the main steps including single-cell and nucleus isolation, separate DNA and RNA capture, and nucleic acid amplification within a single platform. When sequencing the amplified material, this system reduces amplification bias and thus enhances detection sensitivity.

Metabolite detection from minute samples have been a particular strength of DMF systems. Sathyanarayanan *et al.* developed a DMF platform that integrates human liver microsomes immobilized on magnetic beads with a custom-designed, inkjet-printed microheater array to assess individual variations in cytochrome P450 enzyme activity.[87] Fast and parallelized analysis of enzyme activity was monitored using prefluorescent, enzyme-selective model compounds, consuming approximately 15 μg of microsomal protein per assay which is relatively lower compared to conventional assays, making it adaptable for biopsy-scale liver samples. Similarly, Davis *et al.* introduced DMF devices capable of executing and real-time monitoring of multiple ribozymatic cleavage reactions by incorporating fluorescence detection directly in their device design and eliminating the need for traditional gel electrophoresis and enabling rapid,

parallel processing of enzymatic reactions.[88] Mead *et al.* focused on developing a low-cost, open-source DMF platform named OpenDrop for enzyme-linked immunosorbent assay (ELISA) testing, by functionalizing the hydrophobic coating on the DMF device with immobilized capture antibodies on the device's surface, achieving an average water contact angle of 103° for the smooth operation of the assay.[89]

Following the application of ELISA on DMF, **immunoassays** have been a widely studied topic. Insightful analysis and details have been discussed by Su *et al.* recently.[90] Although the mentioned immunoassays do not necessarily incorporate cell culturing in DMF but rather work with the metabolites, they have the potential to be combined with on-chip cell culture in the future. Shamsi *et al.* introduced the first DMF-based electrochemical immunoassay, modifying an ITO top plate to include gold sensing electrodes and silver counter/pseudoreference electrodes, enabling in-line amperometric measurements with a detection limit of 2.4 μl U mL^{-1}, which is falls in the clinical range.[91] Ng *et al.* developed a DMF platform utilizing magnetic forces for particle-based immunoassays, employing antibody-coated paramagnetic particles manipulated by external magnets to facilitate efficient separation and resuspension of thyroid stimulating hormone and 17β-estradiol without the need for oil-based carrier fluids.[92] Hu *et al.* designed a microfluidic immunosensor combining immunomagnetic separation with droplet arrays for the automatic detection of carcinoembryonic antigen and this work achieved high sensitivity and specificity through the integration of magnetic bead-based separation and droplet-based fluorescence detection.[93] Shen *et al.* developed a DMF thermal control chip-based multichannel immunosensor for non-invasive detection of acute myocardial infarction, which was made possible by incorporating precise temperature control and multichannel detection capabilities to enhance assay throughput and reliability.[94] Ng *et al.* developed a portable DMF platform utilizing inkjet-printed cartridges and an integrated instrument to perform ELISA for measles and rubella antibody detection in remote settings.[95]

2.5.2. Metabolite analysis *via* integrated methods. **Impedance** analysis has advanced DMF systems' capabilities in droplet characterization, and cell analysis. Early work by Sadeghi *et al.* introduced a high-sensitivity impedance measurement technique for on-chip droplet characterization, employing a low-voltage, high-frequency signal to accurately determine droplet properties such as size and composition without disrupting droplet integrity.[96] Concurrently, Shih *et al.* developed a DMF platform integrating impedance sensing for real-time monitoring of mammalian cell culture and analysis. This system utilized a parallel-plate configuration with ITO electrodes for the detection of cellular events by measuring impedance changes as cells interacted with the electrodes.[97] Further advancements by Jin *et al.* led to the development of a DMF system with integrated electrochemical impedance detection arrays for multiplexed analysis within a single platform and simultaneous monitoring of multiple analytes.[98] The most recent innovations, such as the one by Zeng *et al.*, have introduced high-frequency, addressable impedance measurement systems capable of operating over a wide frequency range (10 kHz to 60 kHz). This system featured a low-voltage, addressable measurement circuit that enhances sensing accuracy and enables on-site analysis of droplet composition, size, and positioning.[99] The integration of automatic calibration and real-time data analysis ensures high sensitivity and reliability in various applications.

Integration of **mass spectrometry** (**MS**) with DMF has been often employed, as it requires a connection (tube) between two devices. Das *et al.* introduced an on-the-fly MS technique within a DMF platform by incorporating a chip-integrated microspray hole. This method utilized electrostatic spray ionization to analyse sample droplets through a micro hole in the cover plate, enabling real-time monitoring of rapid chemical reactions, such as the seconds-long Hantzsch synthesis.[100] Notably, the analysed droplet remained movable post-MS analysis to enable continuous on-chip analysis. Ruan *et al.* developed a single-cell DMF-MS system employing EWOD for precise manipulation of individual circulating tumour cells[10] by integrating single-CTC isolation and high-performance whole genome amplification within nanolitre droplets for efficient and multiplexed genotyping *via* matrix-assisted laser desorption/ionization time-of-flight MS. Interestingly, Peng *et al.* designed an all-in-one DMF pipeline that automates proteomic sample preparation, including reduction, alkylation, digestion, and isotopic labelling steps.[101] The system integrated thermal control and optimized droplet additives, well-interfacing with high-performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS).[102] This approach enables sensitive analysis of low-abundance proteins, and identifies differentially expressed proteins in cancer cell lines and tissues for proteomic cancer subtyping.

The physical integration of DMF with **surface-enhanced Raman spectroscopy** (**SERS**) is achieved by incorporating SERS-active substrates, such as silver nanoparticles, directly onto the DMF device. Sheng *et al.* developed a method combining micro-Raman spectroscopy with DMF to characterize stress-induced protein particles. Here, microliter volumes of protein solutions on a DMF device generated multiple droplets for Raman analysis.[103] Machine learning models trained on the Raman spectra achieved high classification accuracy (93–100%) in identifying eight types of protein particles. Dong *et al.* integrated DMF with a Translucent Raman Enhancement Stack sensor for *in situ* analysis[104] by handling less than 5 μL of samples with the hydrophilic TRES sensor surface enhancing analyte detection through SERS. Fehse *et al.* presented a reusable DMF-SERS system incorporating a photochemical cleaning step. This design allows for *in situ* SERS monitoring of compounds by

degrading adsorbed analyte molecules *via* ultraviolet irradiation and water droplets.[105]

Integrating DMF with **nuclear magnetic resonance (NMR) spectroscopy** involves positioning micro coils adjacent to the DMF platform to enable direct interaction between the NMR detection field and the microfluidic droplets. In the study by Jenne *et al.*, a vertical-optimized single-sided strip line coil was used to monitor living organisms *in vivo* using 1H-13C two-dimensional NMR.[106] This configuration facilitated the detection of metabolomic changes while DMF supplied oxygenated water to sustain the organisms, effectively preventing anoxic stress. Similarly, Chen *et al.* designed a planar annular microstrip coil tailored to the shape of DMF-manipulated droplets, enhancing detection sensitivity by increasing the filling factor.[107] This setup enabled *in situ* analysis of photoreactions, such as aldehyde–amine and photopolymerization reactions, within the DMF platform.

Field-effect transistor (FET)-based biosensors have also been physically integrated within DMF systems by embedding them into the centre of droplet-driving electrodes. This monolithic integration allows the DMF platform to manipulate droplets containing analytes *via* EWOD and position them precisely over the FET sensors for real-time, label-free electrical detection. Specifically, the system introduced by Choi *et al.* can detect avian influenza antibodies by monitoring changes in the drain current of the FET as antigen–antibody binding occurs.[108] This fully electrical approach eliminates the need for bulky transducers, facilitating an all-in-one chip design compatible with existing fabrication technologies for control and read-out circuitry.

Surface acoustic waves are integrated into DMF devices by fabricating interdigital transducers on piezoelectric substrates, such as lithium niobate ($LiNbO_3$). These IDTs generate SAWs upon electrical excitation, which propagate along the substrate surface. When a droplet is placed on this surface, the SAWs interact with the droplet, inducing internal acoustic streaming and radiation forces that enable precise manipulation of the droplet's position and movement. This method allows for contactless and efficient control of fluids within DMF platforms. Shilton *et al.* demonstrated that SAW-driven heating can rapidly and controllably increase the temperature of microliter-sized water droplets by up to 10 °C within approximately 3 seconds. By adjusting the SAW power excitation profile, this rise time can be further reduced to about 150 milliseconds.[109] In another approach, Zhang *et al.* introduced a DMF device that combines surface wettability gradients with SAWs to enhance droplet control. They created octagonal patterns with wetting gradients and orthogonally distributed IDTs on a $LiNbO_3$ wafer using photolithography.[67] This design allowed for selective manipulation of droplets to facilitate sequential reactions among multiple droplets and improve positional accuracy to the micrometre level.

3. Trends

Recent advancements in DMF have focused on enhancing automation, sensitivity, and integration with analytical techniques. The ability to dynamically and precisely manipulate droplets has enabled real-time monitoring of cellular responses with improved resolution. This real-time capability adds a layer of sophistication to the understanding of cellular dynamics. High-throughput screening has become increasingly viable, as modern DMF platforms facilitate parallel processing, reducing the need for large reagent volumes. Integration with biosensors for analyte detection has expanded the utility of DMF in biomedical research, allowing for rapid, label-free detection of biomolecules.

Another emerging trend is the development of machine learning- and artificial intelligence (AI)-assisted DMF platforms, optimizing droplet manipulation and experimental conditions for more accurate and reproducible assays. These approaches are being used not only to optimize droplet routing and timing but also to learn from experimental outcomes and autonomously adjust experimental protocols in real time. For instance, ML models can predict the success of droplet operations based on device history and environmental conditions, enabling more robust and reproducible assays. AI-driven image analysis is also being used to monitor cell behaviour, detect morphological changes, and guide subsequent droplet manipulations. The scalability of DMF platforms is another facet that propels their utility in cell culture studies, allowing for parallel processing and high-throughput experimentation. The scalability not only optimizes resources but also accelerates data acquisition, streamlining research processes. AI-driven algorithms are now being incorporated to facilitate real-time feedback and predictive control of droplet behaviour. This contributes to closed-loop automation, where the system adapts its parameters based on live sensor data or imaging feedback, thereby improving consistency across experiments. As AI and machine learning techniques continue to integrate into DMF workflows, further advancements in automation, precision, and analytical depth are anticipated, solidifying DMF's role in next-generation biomedical and biotechnological applications. In all these fields, there are only a few examples with cell studies that show the proof of concept of DMF technology.

4. Challenges and opportunities

Despite its advancements, DMF technology faces several limitations that hinder widespread adoption. One major challenge is biofouling, where prolonged exposure to biological samples leads to protein and cell adhesion on the hydrophobic surface, affecting droplet movement. Another issue is droplet evaporation, which impacts long-term culture studies, particularly when working with volatile solvents or prolonged assays. Compatibility with live cells also remains a concern, as prolonged exposure to high voltages in EWOD

actuation can induce stress responses in cells. The integration of DMF with external analytical instruments, such as mass spectrometry and NMR, presents technical challenges in ensuring seamless fluidic interfacing. Additionally, the fabrication of DMF devices requires specialized expertise, limiting accessibility in non-engineering-focused laboratories.

To address these limitations, embedding tissue-mimetic organ-on-chip elements directly into DMF devices can lead a path forward. Incorporating microchannel-based perfusion networks and extracellular matrix coatings within the DMF footprint can dramatically reduce non-specific adsorption and maintain humidified environments that prevent droplet loss. Coaxing flows through microvascular-like channels also means gradually lower actuation voltages can be used, preserving cell viability while enabling higher cell densities in three-dimensional culture. Moreover, modular "plug-and-play" organoid chambers, equipped with built-in sensors for pH, oxygen, and metabolite monitoring, can streamline the connection to downstream analytical tools—sidestepping complex tubing—while standardized chip layouts, microfabrication procedures and cell culture recipes democratize device production. By weaving organ-on-chip architectures into the fabric of DMF, one harnesses automation, miniaturization, and multiplexed control to create truly dynamic, physiologically relevant platforms capable of long-term, high-throughput biological investigation.

5. Conclusions

DMF continues to evolve as a transformative tool for biological and chemical analysis. By offering precise droplet manipulation, real-time monitoring, and miniaturized workflows, DMF systems address key challenges in cell culture, diagnostics, and biochemical assays. While technological advancements in integration and automation are making DMF more accessible, existing challenges in biofouling, evaporation, and long-term stability require further innovation. As research progresses, improvements in materials science, AI-driven automation, and multi-modal sensor integration will drive DMF adoption in both laboratory and clinical settings. In summary, DMF stands as a powerful and versatile tool in the field of cell culture studies, fostering advancements in both fundamental research and practical applications. The advantages including precise control, real-time monitoring, scalability, and integrated functionalities positions DMF as a game-changer, shaping the landscape of cellular studies and propelling the scientific community toward new frontiers of discovery.

Data availability

No primary research results, software or code have been included, and no new data were generated or analysed as part of this review.

Author contributions

Conceptualization, investigation, resources, original draft preparation, review, editing, visualization, project administration, funding acquisition are performed by B. G. The author has read and agreed to the published version of the manuscript.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

The author acknowledges the members of the Biosensors and Devices Lab and BioInterface Science group at Eindhoven University of Technology for fruitful discussions. The author also acknowledges the financial support provided by the Irene Curie fellowship funding by Eindhoven University of Technology.

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