



Droplet microarray platforms for high-throughput drug screening

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Received: 18 March 2023 / Accepted: 15 May 2023 / Published online: 15 June 2023
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Abstract

High-throughput screening platforms are fundamental for the rapid and efficient processing of large amounts of experimental data. Parallelization and miniaturization of experiments are important for improving their cost-effectiveness. The development of miniaturized high-throughput screening platforms is essential in the fields of biotechnology, medicine, and pharmacology. Currently, most laboratories use 96- or 384-well microtiter plates for screening; however, they have disadvantages, such as high reagent and cell consumption, low throughput, and inability to avoid cross-contamination, which need to be further optimized. Droplet microarrays, as novel screening platforms, can effectively avoid these shortcomings. Here, the preparation method of the droplet microarray, method of adding compounds in parallel, and means to read the results are briefly described. Next, the latest research on droplet microarray platforms in biomedicine is presented, including their application in high-throughput culture, cell screening, high-throughput nucleic acid screening, drug development, and individualized medicine. Finally, the challenges and future trends in droplet microarray technology are summarized.

Keywords Droplet microarray · High-throughput screening · Superhydrophobic–superhydrophilic pattern · Surface chemistry

Introduction

High-throughput systems are platforms capable of screening and analyzing a large number of samples simultaneously and are widely used in the pharmaceutical and biotechnology industries as well as in academic research laboratories [1].

Miniaturization is widely used in biological and chemical analyses as a means of high throughput and has great biomedical value [2]. Compatible highly parallel synthesis or the addition of chemical libraries is also essential for achieving high-throughput screening [3]. The implementation of these strategies helps reduce the consumption of compounds, reagents, and cells, reducing the cost of screening in

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industry and academia. For example, the rapid development of genomics, proteomics, and metabolomics in the field of drug development has contributed to the need for sophisticated high-throughput screening (HTS) experiments [4].

Recently, new platforms for HTS have been developed. These systems include microtiter plates, cell-based microarrays, and microfluidic devices; these methods have their own advantages and disadvantages (Table 1). Microtiter plates are a well-established HTS platform that can be performed in 96-, 384-, and 1536-well plate. However, this method consumes 30 to 100 μL of reagents per well, leading to increased reagent expenses and high consumption of cells, and further volume reduction of the microplate is limited [11]. Furthermore, this platform requires multiple pipetting steps and is time-consuming [1]. Thus, a miniaturized HTS platform that can reduce the consumption of compounds and cells and eventually reduce experimental costs is required. Compartmentalization is the biggest bottleneck in HTS; Sabatini and colleagues established a cell-based microarray where they pre-blot the transfection mixture on glass slides and seed the slides with a layer of adherent cells, which are then immersed in a culture medium. This approach has no partitioning between arrays, allowing for high-throughput parallel experimental manipulations; it also reduces cell and reagent consumption and simplifies pipetting steps. However, cross-contamination can occur because of drug molecules in the culture medium [12]. This platform is not compatible with compound screening and is limited to adherent cell cultures [13]. Cell-based microfluidic technology confines the liquids in small channels which reduces the sample consumption and time of experiments and precisely adds the solutions in nanoliter volumes of the devices; this platform has advantages in cell-based assays. However, the microfluidic platform is limited to high-throughput drug screening as the drug input is controlled by proper valves through dedicated channels and leads to high drug loss as well as inaccurate concentration control [11]. Therefore, these platforms are insufficient for biomedical high-throughput screening. In the current biomedical field, there is an urgent need for high-throughput screening technology that simplifies operational steps and maximizes cost-effectiveness.

Droplet microarray (DMA) technology can address the technological barriers in current high-throughput culturing and screening. DMA is a miniaturized high-throughput platform with hydrophilic dot arrays on a superhydrophobic background. The extreme difference in wettability between the hydrophilic dots and the superhydrophobic background facilitates the spontaneous formation of droplet microarrays, which can be used in biomedical applications such as cell culture and screening, and drug testing [3]. Levkin et al. developed a DMA platform for high-throughput screening of live cells in 2D and 3D, including cytotoxicity screening and tumor spheroid screening. In 2020, they designed a DMA

Table 1 Summary of HTS technologies

HTS technologies	Advantages	Disadvantages	Refs
Microtiter plates	Microplate technology is widely used for cell-based screening aimed at basic research and drug discovery; each microwell allows for rapid positioning and time-delayed analysis; the physical walls of the microplate prevent chemical and optical crosstalk between neighboring wells.	Capillary effect forming a physical barrier to the pores preventing further miniaturization of microtiter plates with microliter culture volumes; high consumption of reagents and cells, unfriendly for screening rare, and difficult to expand cells (stem cells, primary cells, etc.); microplate technology requires multiple pipetting steps and dedicated pipetting robots are expensive, increasing experimental costs.	[5, 6]
Microfluidics	Long-term and real-time culture and observation of cell status; the interplay of microfluidic perfusion channels facilitating the integrated analysis of different cell types.	Inability to achieve large compound libraries and high-throughput screening of multiple drug concentrations; limited application of drug toxicity testing.	[7, 8]
Cell-based microarrays	Widely used for phenotypic screening; high-throughput screening in a highly miniaturized format.	Limited to the culture of adherent cells and incompatible with cells in suspension; no separate compartments for cells after inoculation and potential for cross-contamination between sites.	[9, 10]

platform with miniscule volumes (20 nL), establishing an ultrahigh-throughput and miniaturized DMA platform [14]. As a novel miniaturized high-throughput screening platform, DMA plays a crucial role in diverse biological applications and has the following advantages: (a) reduction of compounds and cell consumption, thus lowering the cost of analyses; (b) avoiding cross-contamination between spots; (c) compatibility with the adherent and suspension cells; (d) compatibility with various analyzed techniques to obtain the result; (e) less pipetting manipulation and ultrahigh-throughput screening [14, 15].

In this progress report, we summarize the innovative fabrication and emerging applications of droplet microarrays over the last few years. In the first part of this review, the principles and manufacturing of DMA are summarized. In the second part, the high-throughput screening applications of DMA in cells, nucleic acids, and stem cells, as well as their contribution to personalized medicine, are also presented. We emphasize the potential of the DMA platform for HTS analysis in the biomedical field.

Droplet microarray: design and fabrication

The methods used to fabricate droplet microarrays are constantly being improved and updated, including past wet etching techniques, polydimethylsiloxane (PDMS) stamp methods, and recent innovations such as photo-grafting, thiol-olefin clicking, and nourishment methods using natural materials. In addition, the compound addition techniques and result-reading methods used in the high-throughput screening process of droplet microarrays also played a very important role in the success of the experiments.

Fabrication of droplet microarray

The basic principle of DMA is to create patterned surfaces with immersion differences (Fig. 1A). The advanced development of surface chemistry is of utmost importance for DMA implementation. In past research, many methods have been developed to fabricate DMA. The overall trend and goal of the continuous improvement of these fabrication methods is to simplify the experimental steps, reduce the experimental cost, and create miniaturized high-throughput screening platforms. The transition from hydrophilic-hydrophobic patterned surfaces to superhydrophilic-superhydrophobic patterned surfaces is an important breakthrough in the improvement of DMA fabrication methods.

Fang et al. developed DMA using the wet etching method. First, they prepared hydrophilic silica layers on the surface of silicon wafers by thermal oxidation treatment and made them hydrophobic by silylation. The hydrophobic layer was then coated with photoresist, exposed to ultraviolet light, and removed by wet etching to obtain hydrophilic-hydrophobic

micropatterns. They performed a two-step real-time quantitative PCR assay to measure microRNA after dispensing a 500-nL volume of reaction solution in the hydrophilic zone using a conventional pipette, and the experimental results were reliable and sensitive [21]. Liu et al. developed a DMA platform using the PDMS stamp method (Fig. 1C). They used soft lithography to print PDMS stamps, which were then stamped onto a hydrophilic surface. After the oxygen plasma treatment, nanofilms of hydrophobic PDMS were formed on the contact surface. Their work demonstrates that uniform, highly repeatable microgel arrays can be formed in sizes from 100 to 800 μm , corresponding to thicknesses from 5 to 45 μm , and that this approach also allows for the design of microgels of different shapes including circles, squares, octagons, crosses, triangles, and complex interconnected microgel networks [17]. Ulrich et al. used inkjet printing method to print cell culture medium, L929 cells separately on tissue culture plates while spreading paraffin oil to prevent evaporation, the total reaction volume was 1 μl , and the appearance of spots containing L929 cells after 7 h of incubation was screened using bright field microscopy (10 objective) [22]. However, these devices are based on hydrophilic-hydrophobic patterned surfaces that require complex microfabrication and chemical modifications, such that the fabrication process takes a long time. Individual culture spots are larger than 0.5 μl , which is unfriendly for costly cell screening, limited by the hydrophilicity of the chemical surface, and the proposed further reduction of the spacing between adjacent droplets and the volume of droplets to improve throughput and reduce the volume of individual culture spots is difficult for hydrophilic-hydrophobic patterned surfaces. Therefore, the development of simple, flexible, versatile, and compatible DMA devices remains a significant challenge.

Recently, several new approaches have been developed. The most widely used methods involve the UV-induced decomposition of different coatings to form superhydrophobic interfaces. For example, Guo et al. synthesized dense superhydrophobic TiO_2 layers by FAS-17 modification (which helps reduce the free energy of the TiO_2 surface) (Fig. 1B) and explored and verified the reversibility of wetting under photothermal action; the contact angle of water on the TiO_2 surface was 160°, 3° after UV treatment, and 159° after high-temperature heat treatment. This reversible cycle can be maintained for 30 stability cycles; by using an acoustic droplet injection device, a drug solution with a volume of $\sim\text{pL}$ to $\sim\text{nL}$ can be precisely injected into a 20- μL droplet, and the whole process takes only 20 ms [11]. Levkin et al. developed a lattice-like superhydrophobic pattern. Because UV irradiation on glass plates can trigger radical polymerization for the preparation of superhydrophilic, biocompatible, and transparent nanoporous poly(2-hydroxyethyl methacrylate-co-ethyl dimethacrylate) (HEMA-EDMA)-coated

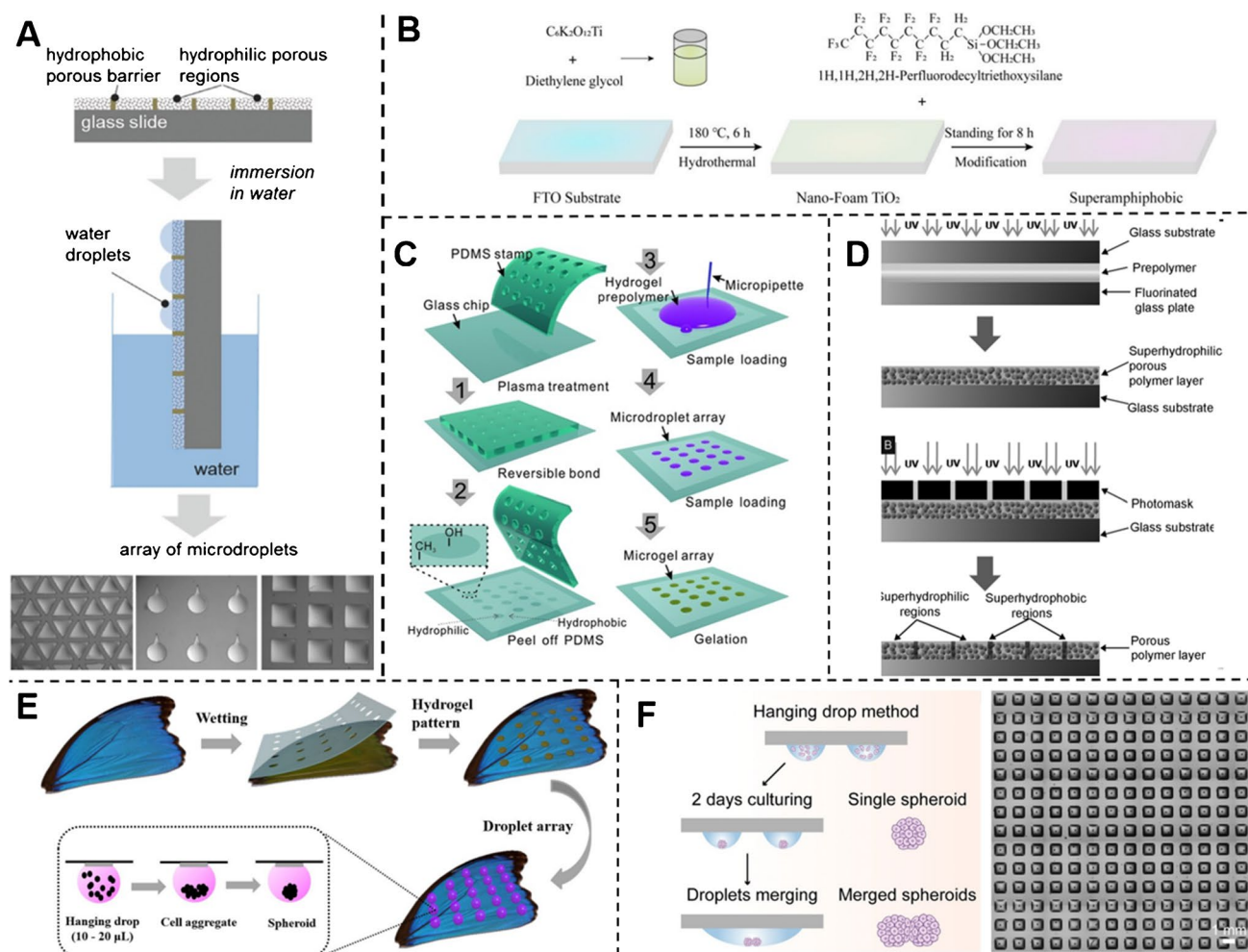


Fig. 1 Methods for fabricating DMA. **A** A schematic diagram of the DMA fundamentals; different surface hydrophilicity [16]. Copyright 2016, Wiley Online Library. **B** A schematic diagram of the synthesis process of a superhydrophobic TiO_2 substrate [11]. Copyright 2021 American Chemical Society. **C** Stamp Method to fabricate DMA [17]. Copyright 2014, John Wiley and Sons. **D** Optical grafting method: (a) formation of super hydrophilic porous polymer films triggered by UV light; (b) fabrication of superhydrophobic lattice-

like patterns by photonic grafting [18]. Copyright 2014, John Wiley and Sons. **E** Preparation of DMA on the wings of natural material large shimmering butterfly [19]. Copyright 2019, American Chemical Society. **F** Schematic diagram of tumor sphere preparation in suspension droplets; programmable assembly of spheres can be achieved by increasing the volume of adjacent droplets to merge droplets [20]. Copyright 2019, Wiley Online Library

films, they produced superhydrophobic lattice-like patterns by UV-induced photo-grafting under precise control of a photomask (Fig. 1D) [18]. They used the rolling droplet method to fabricate DMA by contrasting the extreme wettability of the spots and the background [23]. They also discovered a method based on thiol-alkyne click chemistry that allows the preparation of superhydrophobic and superhydrophilic DMAs quickly and without initiators and showed that the pattern can be applied biologically under aqueous conditions [24]. Zhao et al. developed hydrogel patterns to hang droplets on a bioinspired wing substrate, which was inspired by the hydrophilic peaks and hydrophobic bases of the *Stenocara* beetle. They used *Morpho* butterfly wings as natural superhydrophobic surfaces, and the hydrogel prepolymer solutions

could be infiltrated into them as droplet microarrays, which could be used for 3D cell culture (Fig. 1E). This method is simply fabricated without complex instruments or chemical modifications and can be used in many biomedical fields [19]. To achieve flexible combinations of droplet arrays, Levkin and colleagues programmed the merging of adjacent droplets (proMAD method) by altering the size and distance between the hydrophilic arrays on the DMA and then realized non-contact merging of neighboring cell spheroids in an HTS method, which can be used in many fields of biomedical research, such as cancer invasion, neural development, and cell signaling (Fig. 1F) [20].

On the hydrophilic-hydrophobic patterned surface, the difference in wettability between the hydrophilic and

hydrophobic regions of the droplet microarray is small, which makes further reduction of droplet size in the hydrophilic region hindered ($>0.5 \mu\text{l}$), and compound addition is often performed manually or with specialized liquid handling equipment, which increases screening costs and does not meet the need for miniaturization and high throughput. Studies based on superhydrophilic-superhydrophobic patterned surfaces have greatly improved these drawbacks, as the droplet size is greatly reduced (10–200 nL), and the great difference in hydrophilicity allows spontaneous droplet formation in the superhydrophilic region. However, as droplet volume decreases, droplets can easily evaporate if no action is taken, and many studies have been conducted to avoid evaporation problems by increasing the humidity of the culture dish [5, 14, 15]. Another option to prevent evaporation is to cover the droplets with oil; Ulrich et al. used mineral oil or similar materials as a barrier to protect submerged cell cultures A41; Bruno et al. used the hygroscopic properties of glycerol to maintain a constant water content in the patches by adding 30% glycerol to the deposited liquid to optimise the rheological properties of the droplets and to solve the evaporation problem [25], as manufacturing techniques continue to improve and update facilitating the development of patterned droplet arrays, making them of increasing interest to scientists as miniaturized, high-throughput screening tools.

Techniques for adding compounds into DMA

Techniques for adding drugs to DMA are the most critical steps for chemical or biological high-throughput screening and toxicity testing. Every array is a microreactor, and different compounds must be added to the droplets, which is a complex process. In this section, we summarize methods for adding chemical agents to individual microdroplets (Table 2).

Immediate drop-on-demand technology

Immediate drop-on-demand technology (I-DOT) is a method for handling trace amounts of liquids in the nanoliter to microliter range using non-contact pressure injection technology, which allows for flexible and contactless dispensing of liquids or cells without the risk of cross-contamination (Fig. 2A). The basic working method of I-DOT is the

ejection of liquid from the bottom nozzle by a short pulse of compressed air [29, 30]. Popova et al. cultured single cancer spheroids on the DMA platform within nanoliter volumes, using the I-DOT device to dispense cells into the arrays. In order to make a comparison, they also performed manual cell inoculation; the outcome revealed that they formed cell spheroids successfully (including MCF-7, HEK293, and HeLa cells). The size of the sphere depends on the initial number of cells in the droplet, and the size distribution of the spheres on the DMA spots was consistent with the Poisson distribution and comparable between manual and automatic seeding methods. This means that the I-DOT device has satisfactory performance in the distribution of cells on DMA. Subsequently, they used I-DOT to stain the spheroids and added the drug to the spheroids for the next experiment [7].

Sandwiching method

The sandwiching method for adding compounds into the droplets has advantages in parallel addition reagents, using a non-contact printer to preprint a library microarray and then parallel-adding to the droplets by a very simple handheld device, while other parallel treatments, such as staining, can be performed using the same devices. Levkin et al. printed the fluorescently labeled dye on a microscope glass slide using the non-contact liquid dispenser; the DMA slide containing embryos and the glass slide containing coated compounds were sandwiched together by an aligner, indicating that using the sandwiching method for the parallel addition of different compounds to arrays is workable without any cross-contamination between adjacent droplets (Fig. 2B) [27]. However, 50% of the volume will be reserved on the library microarray slide after the microreactor, which results in the wasting of reagents [15]. Levkin et al. proposed a reverse transfection and reverse small-molecule drug screening method by pre-printing the drug to be screened on a DMA plate and forming the droplet-microarray by the “rolling droplet” method (Fig. 2C). A live cell microarray platform that combines parallelization, partitioning, and miniaturization has been realized. In the future, it is believed that it will be possible to purchase custom preprinted chemical libraries for DMA slides [5].

Table 2 Comparison of techniques for adding compounds to DMA

Techniques	I-DOT	Sandwich method	Acoustic droplet-assisted
Object	Drugs, dyes, cells	Drugs, dyes	Drugs, dyes
State of the added object	8~50nL droplets	Array of compound droplets after drying	pL~nL droplets
Reusability	Yes	No	Yes
Whether to contact DMA	No	Yes	No
Flexibility	High	Low	High
Adjustable droplet size	Yes	Yes	Yes

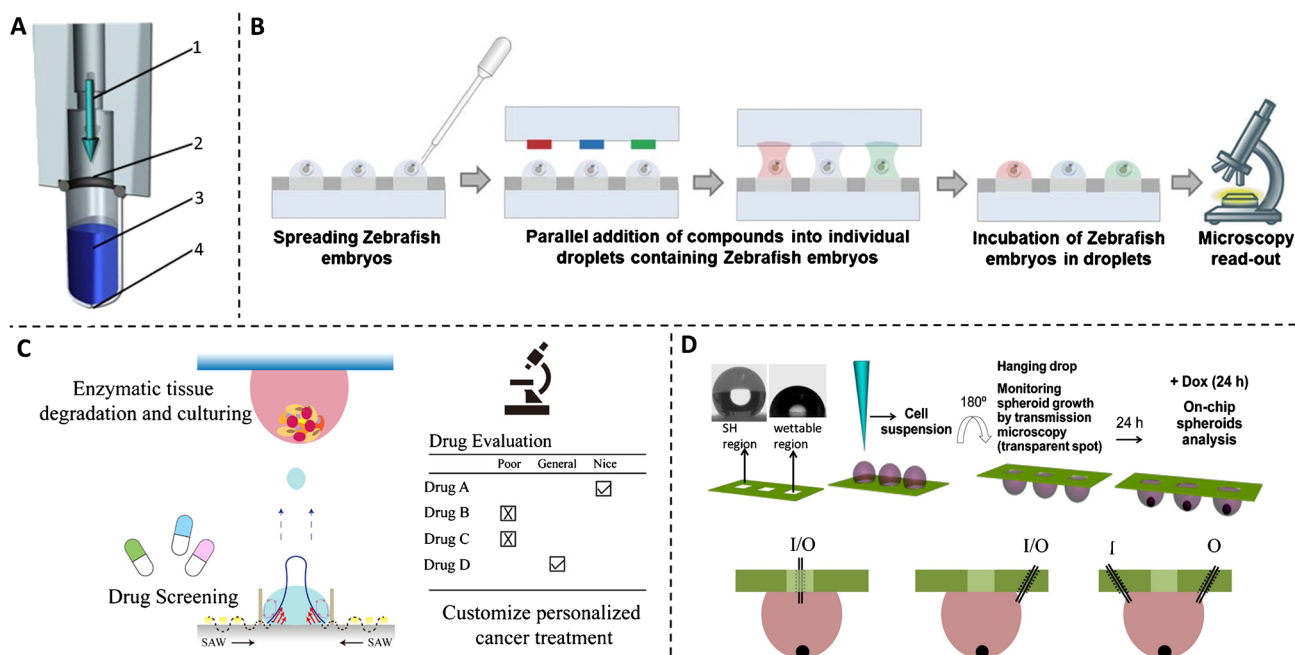


Fig. 2 Methods for adding compounds to droplets. **A** Schematic diagram of I-DOT: (1) compressed air pulse, (2) seal ring, (3) source well filled with liquid, (4) nozzle, (5) droplet [26]. Copyright 2015, ELSEVIER. **B** Sandwich method of adding compounds [27]. Copy-

right 2019, Wiley Online Library. **C** An acoustic droplet-assisted method for adding drugs to droplets of cultured cells [11]. Copyright 2019, American Chemical Society. **D** Compound addition to droplets by perforation [28]. Copyright 2014, American Chemical Society

Innovative methods for adding compounds to DMA:

Guo et al. developed an acoustic droplet-assisted superhydrophilic superhydrophobic platform (Fig. 2D) that can inject a drug solution into the spots precisely in 20 ms (one print), and the volume could vary from pL to nL, which can significantly reduce drug consumption. They validated this platform by culturing and screening colon cancer pathological tissues from five patients. The screened outcomes of multiple drugs at different concentrations of tumor cells in different patients were consistent with the pathological information of the patients after the actual medication [11]. Mano et al. proposed superhydrophobic chips for high-throughput cell spheroid generation and drug screening [28]. To add and remove media directly from the spot, they perforated the inside of the hydrophilic area of the array. However, the location of the perforation in the hydrophilic location is prone to evaporation and contamination of droplets, so they chose to perforate the superhydrophobic area 1 mm away from the hydrophilic area, which can be used laterally for media exchange. Another advantage of perforating the superhydrophobic area is that two adjacent ports can be used as a pair of imports and exports for the dynamic exchange of media (Fig. 2E).

Techniques for result reading method

The problem that needs to be solved determines the way the results will be read. As a platform for high-throughput screening, the droplet microarray as an in vitro screening system has relatively more advantages in cell culture compared with traditional experimental animal models. The main read-out method for the analysis of DMA results is based on optical techniques, and microscopy is the most widespread tool in the analysis. Most DMA plates are made of transparent materials, such as glass and PDMS, which is the basic condition for obtaining microscope readings. Cells can also be stained with a fluorescent stain to assess their status. However, microscope-based readings often require specific markers. As a complement, label-free readout methods have been developed in recent years.

As a tool to detect cellular functions, fluorescence microscopy plays an essential role in DMA for high-throughput screening in the assessment of cell viability and morphology. Evaluation of cell morphology and viability in screening experiments using the DMA platform for single cells, cell spheres, blastomeres, and stem cells is a vital source of data. Cell viability of the droplets was evaluated by live/dead staining. In many studies, calcein AM and propidium iodide (PI) are typically used to assess cell viability. Calcein AM is

a fluorescent esterase substrate that can be hydrolyzed to the green fluorescent product (calcein) in living cells, which is an indicator of active cellular metabolism to retain the esterase product. PI readily passes through damaged cell membranes and binds to DNA, but cannot pass through intact cell membranes; hence, red fluorescence shows dead cells. After staining the cells, fluorescent images are obtained using a microscope to determine the cell viability parameter, which is calculated by dividing the calcein AM-positive area by the sum of calcein AM- and PI-positive areas [13].

Optical methods usually require fluorophore markers; however, to understand how drug therapy affects the composition and modification of cellular biomolecules, label-free readout methods can be used as supplements. Plumere et al. developed an electrochemical microdroplet array (eDMA platform), which has a three-electrode system and avoids cross-contamination. The eDMA platform can monitor the molecules consumed and products generated by living cells directly and continuously. At the same time, the biological environment of DMA is not affected. Two sets of parallel Au bands were used as the pseudo reference electrode (pRE) and counter electrodes (CE), and another set of Au bands was used as the working electrodes, which were vertically placed on the CE or pRE. A layer of chemically functionalized permeable porous polymethacrylate covered the three electrodes and formed hydrophilic fields that were separated by 150- μm -wide hydrophobic ones [15]. Each hydrophilic reaction forms an electrochemical cell array, which meets the requirements of standard electrochemical methods; redox-active analytes can be electrochemically detected and quantified by diffusion through the hydrophilic region, demonstrating the broad applicability of the eDMA platform [15, 31]. In recent years, matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (MS) has become a major tool for cell-based high-throughput drug discovery and analysis, enabling rapid spatially resolved analysis of individual cells in a population by MS imaging [32]. In addition, the application value of MALDI-MS in single-cell metabolite analysis has been demonstrated, and it can analyze changes in the biomolecular composition of whole cells and identify the pharmacodynamic (PD) effect of the cell [31]. Levkin et al. proposed the droplet microarray–mass spectrometry imaging platform, which can achieve cell-based compound high-throughput screening in nanoliter- or microliter-scale volumes and measure the PD markers in arrays directly.

Applications of droplet microarray

DMA has unique advantages in the biomedical field because of its miniaturization, high throughput, low sample consumption, and open reaction environment that is compatible with multiple analytical methods. There have been numerous studies on DMA applications in recent years (Table 3).

Droplet microarrays for high-throughput cell culture

Phenotype-based high-throughput screening of live cells is crucial in drug development and toxicology studies. However, previous cell-based analytical platforms are usually in microtiter plates in 96- to 1536-well format; screening based on these platforms consumes more reagents and cells. Owing to the increasing cost of experiments and the increase in studies based on rare cells, such as primary and stem cells, there is an urgent need to design miniaturized high-throughput screening platforms. In this section, we outline the application of DMA platforms for adherent and suspended cells, single-cell analysis, and 3D cell sphere screening.

Adherent and suspended cells in droplet microarrays

Cell-based microarrays are an effective tool for high-throughput experiments; however, they can only be used for adherent cell-related analysis, and there are difficulties for non-adherent cell cultures. These include immune cells and other blood cells, stem cells, cancer cell lines, and others [40], mainly due to the easy loss of cells during washing, staining, and other processes, as well as the challenges of cell analysis imaging. In recent years, droplet microarrays have become a powerful platform for the high-throughput culture and screening of suspended cells.

Levkin et al. developed droplet microarrays based on super-hydrophilic-superhydrophobic patterning to form droplets containing Jurkat cells and stain and fix them in one step without loss of droplet content. A semi-automated working model for the automated imaging and analysis of cells suspended in droplets was developed. The system was tested by introducing Jurkat cells into an antitumor drug, demonstrating the potential of the system for suspension cell culture [9]. They later cultured embryonic stem cells in suspension droplets, which formed and differentiated into germinal bodies with three germinal layer cells, and 774 FDA-approved drugs were screened, resulting in 84 drugs that significantly inhibited germinal body formation and 50 compounds that affected one or more measured parameters, based on measurements of germinal body size, roundness, toxicity, and dryness. All the above experiments demonstrate the potential of droplet microarrays for the culture of suspended cells and help advance relevant developments in biomedicine [4].

Their research is also applicable to adherent cells, allowing for the 24-h culture of adherent cells, drug treatment, and gene overexpression in a droplet microarray platform. The platform reduces reagent and cell consumption, allows parallel and simultaneous inoculation of cells and addition of compounds without cross-contamination, and is compatible with standard microscopes for a variety of readout results [23].

Table 3 Summary of research on droplet microarrays in recent years

Application	Method for DMA fabrication	Method for adding compounds to DMA	Readout method	Readout analysis	Volume of reagent	Cells	Refs
2022 “Cells-to-cDNA on Chip” for phenotypic assessment and gene expression analysis.	Grid-like superhydrophobic pattern on the superhydrophilic HEMA-EDMA film fabricated by photo grafting via the UV-induced thiol-yne click reaction.	Pipetted and nanoliter volume dispenser	Microscopy	Bright-field microscope images; fluorescence microscopy images	200 nL–5 μ L	1–1000	[33]
2022 Screening identifies proteins for maintaining pluripotency of hiPSCs by using DMA.	DMA was purchased from Aquarray GmbH (Eggenstein-Leopoldshafen, Germany).	/	Microscopy	Live/dead staining; immunofluorescence staining	200 nL	/	[34]
2021 Formation and screening of tumor spheroids.	Grid-like superhydrophobic pattern on the superhydrophilic HEMA-EDMA film fabricated by photo grafting via the UV-induced thiol-yne click reaction.	I-DOT dispenser	Microscopy	Live/dead staining of Spheroids	100 nL	150	[7]
2021 Acoustic-assisted DMA platform for high-throughput screening of patient-derived tumor spheroids.	Super hydrophilic spots obtained by irradiating the FAS-modified superhydrophobic TiO ₂ substrate with a 40-W UV lamp (366 nm) for 15 min using a patterned mask.	Acoustic droplet ejecting technology	Microscopy	Live/dead staining; bright-field microscope imaging	20–30 μ L	1000	[11]
2021 Drug sensitivity and resistance test on primary patient-derived chronic lymphocytic leukemia cells by DMA platform.	DMA was purchased from Aquarray GmbH (Eggenstein-Leopoldshafen, Germany).	I-DOT dispenser	Microscopy	Live/dead staining	100 nL	100	[35]
2021 DMA enables short-term culture and xenotransplantation high-throughput screenings of hiPSCs.	DMA was purchased from Aquarray GmbH (Eggenstein-Leopoldshafen, Germany).	/	Microscopy	Live/dead staining Immunofluorescence staining	200 nL	/	[13]
2021 High-throughput cell-based assays.	DMAs were prepared on transparent indium tin oxide (ITO)-coated slides.	I-DOT dispenser	Microscopy; MALDI-MS	Live/dead staining; MALDI imaging analysis	40 nL	10	[32]

Table 3 (continued)

Application	Method for DMA fabrication	Method for adding compounds to DMA	Readout method	Readout analysis	Volume of reagent	Cells	Refs
2021 Demonstrate that the morphology, viability, and gene expression patterns of cells cultured on DMA are comparable to those of standard cell culture.	HEMA-EDMA polymer, poly(thioether) dendrimer coatings, and surface patterning were purchased from Sigma-Aldrich Chemie GmbH, Germany, and Merck Chemicals GmbH, Germany.	/	Microscopy; next-generation sequencing;	Actin staining; transcriptomic landscape; GO analysis; analysis of HeLa-CCL2 transcriptome post culture	3 nL; 9 nL; 100 nL	/	[6]
2021 High-throughput screening of drug-eluting coatings on DMA based on ultrasonic spray deposition	PDMS, PMMA, and dichloromethane (DCM) were mixed together and sprayed on the slides (superhydrophobic surfaces), which were covered with a metal mask to form relatively hydrophilic spots by spraying solutions of coating materials.	Ultrasonic spray deposition	Scanning electron microscopy; camera; inverted microscope; smartphone	Image analysis	/	/	[36]
2020 Detection of multiple HIV retroviral nucleic acids by DMA based on nanosensing probe patterns.	Establishment of the MoS ₂ -DMA FRET sensing platform	Non-contact liquid dispenser	Fluorescence microscopy	Average fluorescence intensity	<150 nL	/	[37]
2020 The DMA platform for combinatorial solid-phase synthesis of over 500 different compounds and analysis.	Grid-like superhydrophobic pattern on the super hydrophilic HEMA-EDMA film fabricated by photo grafting via the UV-induced thiolene click reaction.	I-DOT dispenser	Raman microscopy; microscope;	MALDI-TOF-(MS) analysis; live/dead staining; electrospray ionization (ESI)-MS	100 nL	/	[3]
2020 DMA platform for the formation of microarrays of bacteria for high-throughput drug screening.	DMA was purchased from Aquarray GmbH.	Sandwiching methods	Microscopy	Active cell staining	90 nL	/	[38]
2020 Self-splitting of droplets by using patterned adhesive surfaces for separation and multi-detection of microdroplets.	Droplet self-splitting into a microdroplet array by impacting on a patterned adhesive surface	/	High-speed camera	Imaging	/	/	[39]

Table 3 (continued)

Application	Method for DMA fabrication	Method for adding compounds to DMA	Readout method	Readout analysis	Volume of reagent	Cells	Refs
2020 Effect of 774 FDA-approved drugs on the transfection efficiency of CHO-K1, Jurkat, and HEK293T cells using the DMA platform.	The DMA slides were purchased from Aquarray GmbH.	I-DOT	Microscope	Cell viability; transfection efficiency	20 nL	/	[14]

Single-cell analysis in droplet microarray

Single-cell analysis (SCA) reveals complex reactions in the biomedical field, and its main goal is to study biological problems at single-cell resolution. In terms of studying heterogeneity within cell populations, most clinical diagnoses and treatments are currently based on large-cell populations, although some studies have shown heterogeneity within cell populations. SCA identifies unique biomarkers expressed by individual cells in heterogeneous cell populations and provides essential information regarding the response of individual cells to different environmental cues. For example, it is an indicator of patient-specific retention of malignancy. Another important application of SCA is the identification of rare cells such as stem cells and circulating tumor cells, which are important for disease diagnosis and treatment. In recent years, with the continuous progress of science and technology, single-cell analysis has rapidly developed in the biomedical field, but there are still limitations such as low cell viability and easy loss of trace cellular nucleic acids and proteins during isolation. Therefore, there is an urgent need to develop new techniques for SCA research [41, 42].

In the last decade, the latest technologies used for SCA research have mainly included microfluidic chips and microplate-based methods, flow cytometry, and automated cell collection using capillaries, magnets, electric fields, or perforated probes. The basic requirement for each of these methods is that they must be fast, efficient, and gentle to collect isolates and high-quality single cells that maintain their natural expression profile [43]. Despite the progress of these methods in updating single-cell research methods, they still face many challenges. Microfluidic platforms rely on flow control systems that do not provide a suitable physiological environment for cells and are unfriendly to adherent cells [44, 45]. Reagent consumption and inability to index are equally problematic [46]. With these factors in mind, scientists are working to establish platforms that are more suitable for culturing and analyzing individual cells. In the superhydrophilic-superhydrophobic DMA-based platform, cells are cultured in nanoliter-sized droplets, a miniaturized volume that is more conducive to culturing individual cells and enables rapid cell localization. No cross-reactivity or contamination was observed between the cell culture media. This ensures that individual cells are not affected by other cells and that specific nutritional support and growth factors can be provided to the cells. Thus, the DMA platform can be used for single-cell analysis. Levkin et al. optimized the distribution and culture conditions of individual cells on the DMA platform using the finite dilution method (Fig. 3A). Their study showed that single cells cultured on the DMA platform have a 100% survival rate and cell proliferation rate close to that of traditional culture methods, which can achieve multi-step operation of pipetting-free diffusion of

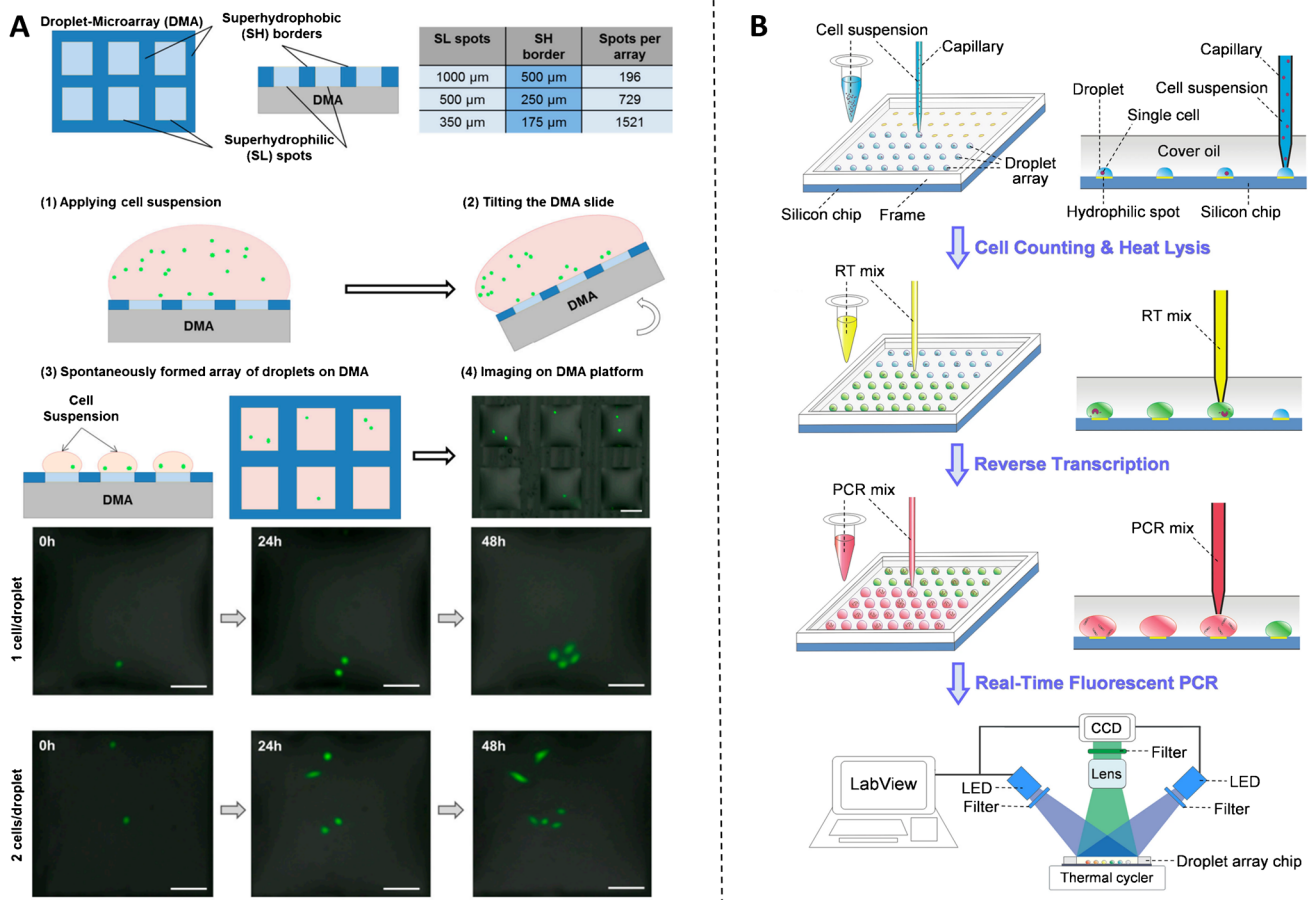


Fig. 3 Droplet microarrays for single-cell analysis. **A** (1) The droplet size and its corresponding number of spots; (2) a schematic diagram of single-cell droplet array formation [47]. Copyright 2016, MDPI. **B**

DMA platform for single cell sequestration, lysis, reverse transcription, and quantitative PCR analysis [48]. Copyright 2015, MDPI

cells, staining, fixation, microscopic analysis, and favorable tools for single-cell analysis [47]. Zhu et al. developed an ml-volume-based DMA platform that allows the quantitative determination of gene expression in individual cells. The entire process of cell encapsulation, lysis, reverse transcription, quantitative PCR, and real-time fluorescence detection was performed on the same droplet (Fig. 3B). They optimized the volume concentration of the droplets of cells to obtain the optimal single-cell occupancy ratio; liquid manipulation was performed by the droplet robot; the fluorescence intensity images were obtained for analysis [48]. Salehi-R et al. designed and developed addressable oil-encapsulated droplet microarrays for single-cell protein determination of the tumor suppressor protein p53 using an antibody sandwich assay and investigated the role of P53 in chemotherapeutic drug response in a simple and rapid procedure that involves loading single cells, lysing them, and measuring protein expression using total internal reflection fluorescence [49]. From the above study, it can be seen that DMA has great potential in the field of single-cell analysis, whether

for single-cell culture, quantitative nucleic acid detection, or protein analysis.

3D cell spheroids screening in droplet microarray

Cell-based phenotypic screening is of paramount importance in areas such as drug development. Swinney et al. reported that 56% of small-molecule drugs approved between 1999 and 2008 were discovered through cell-based phenotypic screening [50]. Cell-based phenotypic screening approaches identify modulators through an overall regulatory feedback mechanism, in the absence of a relevant direct molecular target context [51]. Although a large number of chemotherapeutic oncology drugs have been screened using this approach, they are not always available for clinical use. One of the major reasons for this is that most of the screens are based on 2D tumor models [11]. 2D models do not show the drug metabolism, proliferation, and differentiation functions of cells due to the lack of an extracellular matrix and the real structure of tumor models.

In recent years, the rapid development of 3D cell culture technology has been of great significance for *in vitro* tumor models. 3D models can simulate physical and biochemical microenvironments that are more similar to the real structure of tumors; thus, the response to drugs is more similar to the *in vivo* response [7, 11, 21]. 3D tumor models can compensate for the shortcomings of 2D models and have become the mainstream platform for cell screening. Currently, the major 3D cell sphere fabrication methods include microfluidic devices [52], microtiter plates, and 3D scaffolds. However, these methods still have limitations; for example, the technology of microfluidics is not compatible with high-throughput screening, and the method of microtiter plates has high reagent, compound, and cell consumption and is not suitable for rare cell poetry selection [53]. Therefore, it is necessary to develop a high-throughput, miniaturized 3D cell culture model. Cell spheroids can be used as a model for building *in vitro* organs and as a potential link to the gap between monolayer culture and animal model studies. The most commonly used tumor cell spheroids resemble aggregates without artificial attachment mechanisms. First, it has been shown that this cellular structure resembles the microenvironment of tumor cells *in vivo*, including oxygen concentration, pH, and growth factors [54, 55]. Second, cell spheroids can mimic natural tumor tissue structures to the maximum extent, such as living spheroids and necrotic cores.

The droplet technique is favorable for producing tumor spheroids (Fig. 4A, B). The superhydrophilic array of spotted culture zones and superhydrophobic background allows each culture medium to not interfere with each other, the spheroid size can be controlled according to the initial cell number, and flipping the culture plate after droplet generation can take advantage of gravity to form

cell spheroids [20]. Mano et al. inoculated suspensions with different densities and cell types (L929 and SaOs-2) on hydrophilic spots of the DMA platform. The efficacy of a commonly used clinical cytostatic agent (adriamycin–Dox) was tested after cell sphere formation. The suitability of the DMA platform for direct drug screening using a tumor cell sphere model has been demonstrated [28]. Levkin et al. used a droplet microarray platform to form spheroids of several common tumor cells in the form of 100-nL droplets in 24 to 48 h, with 150 cells per spheroid, up to 7 days of survival, and over 90% survival, with volume varying with the initial cell count. They treated HeLa cell spheroids with drugs and demonstrated a clear dose response of the platform to three different anticancer compounds [7].

Droplet microarray for stem cell screening

Pluripotent stem cells, such as embryonic stem cells and induced pluripotent stem cells (iPSCs), form all cells of the germ layer, except for extra-embryonic structures such as the placenta [56]. Because pluripotent stem cells can differentiate into any cell type in the body and have an unlimited capacity for self-renewal, the culture and use of pluripotent stem cells hold great promise in the field of regenerative medicine and have the potential to revolutionize basic and clinical biomedical science [57]. However, difficulties remain in culturing pluripotent stem cells *in vitro* to maintain their self-renewal capacity and inhibit spontaneous differentiation, making the *in vitro* high-throughput screening of stem cells challenging. This subsection introduces the application of the DMA platform in culturing and screening embryonic stem cells and human-induced pluripotent stem cells.

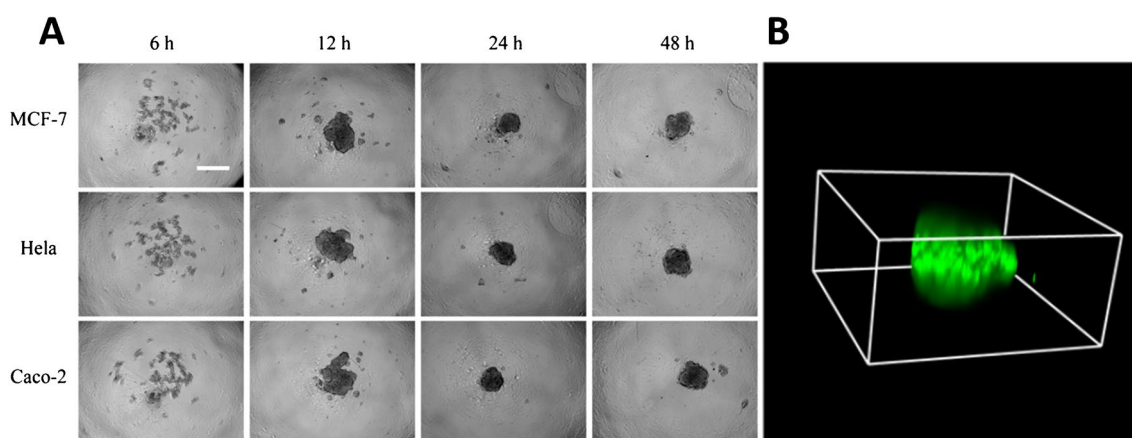


Fig. 4 Formation and culture of tumor spheroids on DMA. **A** Microscope imaging of MCF-7, HeLa, and Caco-2 cells after culture for 6, 12, 24, and 48 h (scale bar = 100 μ m). **B** Fluorescent 3D modeling of tumor spheres [11]. Copyright 2019, American Chemical Society

Droplet microarray for iPSCs

The iPSC-like organoids are structurally and functionally similar to *in vivo* organoids and are re-edited from adult somatic cells, so there are no ethical issues or immune rejection reactions using patient-specific induced pluripotent stem cells. Therefore, iPSCs have great potential for clinical applications in the fields of tissue engineering, disease modeling, drug development, and personalized medicine. For example, iPSC from patients with monogenic diseases can be used to construct *in vitro* models that are useful for understanding the pathological mechanisms of the disease and for drug screening [58]. Therefore, to use iPSCs for industrial medical applications, such as drug screening and regenerative medicine, *in vitro* culture technology must be improved to meet the requirements of safety, efficacy, convenience, high throughput, and cost-effectiveness.

Early methods of culturing iPSCs involved co-culture with feeder cells in media containing fetal calf serum (FCS). The feeder cells were prepared from irradiated or mitomycin-C-treated mouse embryonic fibroblasts (MEFs). Gelatin is a deformed hydrolyzed collagen and feeder cell, and FCS provides the attachment factor together to form a culture system for iPSCs; however, FCS feeder cells contain heterozygous mutagens of uncertain composition, causing multiple problems for the group in culture, for example, cell cross-contamination, long preparation time, and inability to achieve high-throughput screening. Currently, iPSCs are mainly cultured on Matrigel (MG), an animal-derived basement membrane extract, which hinders the use of iPSCs in biomedical applications because it is an animal-derived material with different physical and biochemical properties depending on the batch [59]. Recently, the development of more advanced culture media for iPSCs has become a definite trend. Containing high-purity laminin, fibronectin, and yolk adhesion proteins, extracellular matrices do not contain xenogeneic cells, but they are also not suitable for use with iPSCs in industrial regenerative medicine and drug development because they are costly and time-consuming to prepare [60]. The use of synthetics instead of feeder cells or extracellular matrix proteins, as mentioned above, can effectively avoid cross-contamination and alloimmune reactions caused by animal-derived products, improve the reproducibility of experiments, and reduce the cost of cell culture.

Because iPSCs can be used to construct think-off models for various diseases, they have great potential for high-throughput drug screening of diseases; however, their spontaneous differentiation and low value-added rate during *in vitro* culture are considerable obstacles to achieving this purpose. Therefore, it is of great value to develop a platform with low cell and reagent consumption and high throughput for culturing and screening iPSCs. The surface physical properties of the medium, such as topography, affect the self-renewal and spontaneous differentiation of iPSCs [61]. Levkin et al. demonstrated that the DMA platform could be

used to culture iPSCs without MG for adhesion, with 200 nL per fluid, 672 spots per microscope slide, and 24 h of culture without differentiation. Furthermore, they showed that cells grown in small volumes maintained better pluripotency than human induced pluripotent stem cells (hiPSCs) cultured in a 2-mL cell medium, demonstrating that DMA can be used as a high-throughput screening platform for hiPSCs without receiving animal-derived material to influence the results [13]. Levkin et al. recently performed experiments on 231 combinations of 11 proteins and screened 10 proteomic coatings for significantly increased expression of NANOG (an indicator of pluripotency) compared with that of matrix gel coatings (Fig. 5A), identifying two coatings (BIK (EphB4+DAG1+LN 521) and GHK (EpCAM+BSG+LN 511)) that maintained long-term pluripotency and differentiated into three germ layers (Fig. 5B) [34].

Droplet microarray for ESCs

Human embryonic stem cells are inner cell mass cells removed from blastocysts for culture. When induced with specific molecules, they differentiate into various cell types that can be used to address a variety of clinically challenging diseases, such as restoration of islet cell function in diabetic patients, cancer treatment, injury repair, and reversal of degenerative diseases. Therefore, it is important to build a mature *in vitro* embryonic stem cell culture platform [63].

Since stem cells are susceptible to various environments, such as the extracellular matrix and intercellular interactions, extensive research has been conducted to simulate a more natural cellular microenvironment. Currently, there are challenges in culturing embryonic stem cells, maintaining their proliferative capacity, and inhibiting their self-differentiation. For example, mouse embryonic stem cells need to be passaged periodically and provided with leukemia inhibitory factors [64]. Second, the culture process requires constant stimulation of cell attachment to the surface, which is usually performed using MEF and gelatin coating. MEF secretes soluble cytokines and maintains the stemness of stem cells; however, its acquisition and removal process can have an impact on stem cell culture, so the current method of stem cell culture is not optimal.

Levkin et al. showed that the micro-nano surface morphology of HEMA-EDMA can inhibit the differentiation of embryonic stem cells and maintain the stemness of embryonic stem cells without the need for MEF [65]. On this basis, they developed a DMA platform for *in vitro* high-throughput culture of embryonic stem cells using a superhydrophobic-hydrophilic micropatterned DMA platform that can save up to 1000-fold in volume and compound cost, making *in vitro* high-throughput culture of embryonic stem cells possible (Fig. 5C) [62]. Embryoid bodies (EBs) have been used as models for the *in vitro* differentiation of pluripotent stem cells for 50 years [66]. Tronser et al. developed a DMA

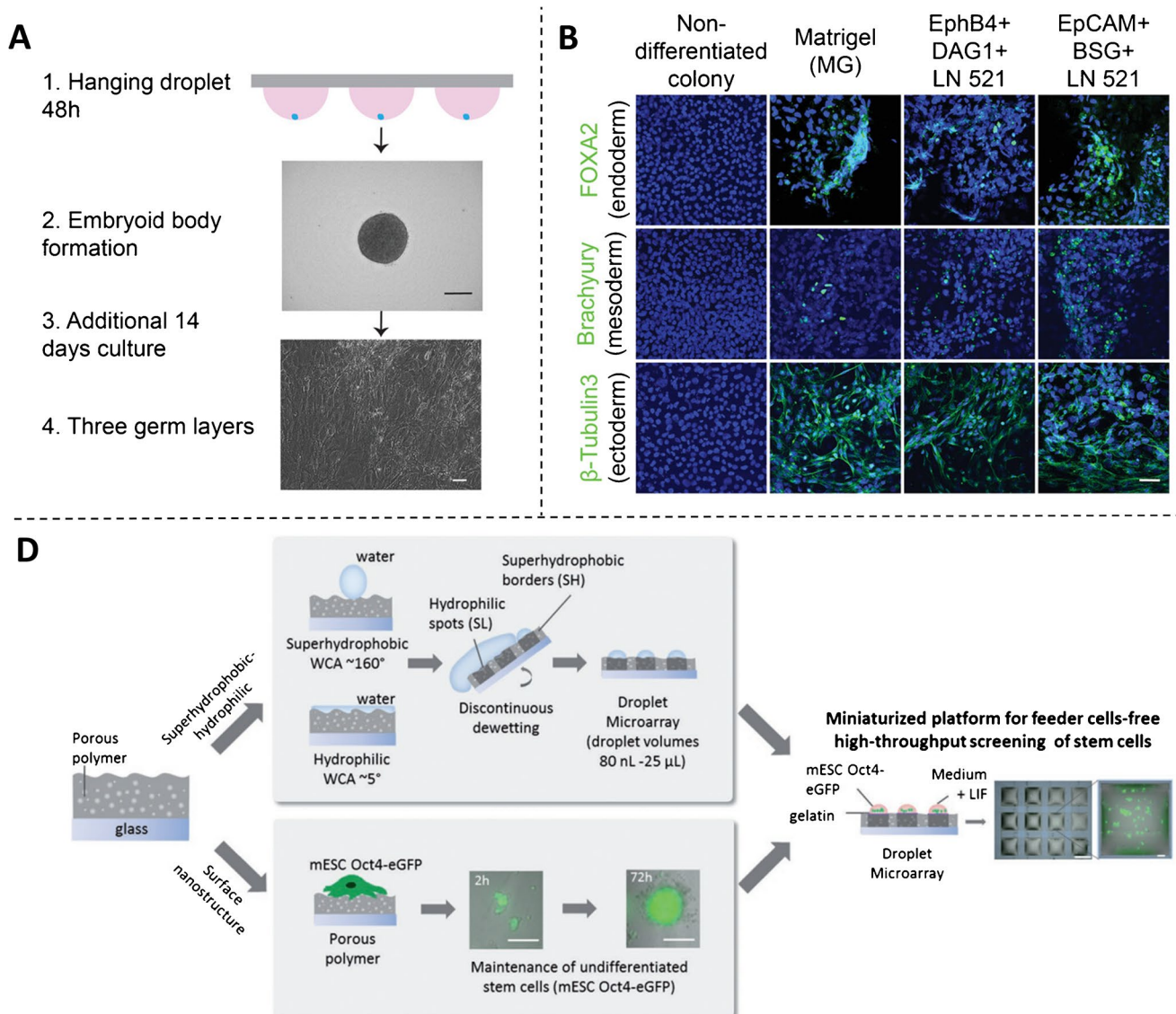


Fig. 5 Schematic diagram of differentiation of hiPSCs into three germ layers. **A** Flow chart of differentiation of hiPSCs into three germ layers. Copyright 2022, Wiley Online Library [34]. **B** Schematic diagram of IF staining results of markers for endoderm, mesoderm, and ectoderm in non-differentiated colonies (NC) and MG-, BIK-, and GHK-coated surfaces. Scale bar: 20 μm [34]. Copyright

2022, Wiley Online Library. **C** The versatility of superhydrophobic-hydrophilic nanoporous polymer microarrays for culturing embryonic stem cells is illustrated: controlled volumes (80 nL–25 μL) can be formed spontaneously; porous polymer surface roughness helps maintain stem cell stemness [62]. Copyright 2017, Wiley Online Library

platform for high-throughput EB suspension culture, taking advantage of the DMA platform to enable suspension cell culture. This platform streamlines the cumbersome steps of traditional EB implementation techniques and reduces reagent and cell consumption during the culture process. They characterized the size, roundness, and distribution of EBs and used this platform to screen 774 FDA-approved compounds, including mycophenolate mofetil, which is developmentally toxic, and eptifibatid, which is embryonically lethal. The DMA platform can be valuable in biomedical fields such as drug development [4].

Droplet microarray in nucleic acid screening

Infectious diseases have a profound impact on the progress of human civilization, not only in terms of health challenges but also the course of war and even the fate of a nation. Unpredictability and potential global explosiveness are the most essential characteristics of infectious diseases [67]. Therefore, rapid detection and treatment of infectious diseases are essential to control the source of infection.

For a long time, the culture of microorganisms and biochemical assays has required large basic laboratory facilities.

For some pathogens, the host needs time to develop an immune response, thus preventing early detection of pathogenic infections, such as human immunodeficiency virus (HIV). Therefore, molecular diagnostic tools are becoming increasingly important for the diagnosis of pathogens [53]. Current molecular assays are time-consuming and require specialized technicians and large equipment. Therefore, it is critical to develop a new nucleic acid detection platform that is highly sensitive, rapid, compatible with low sample/reagent consumption, and capable of performing multiplexed assays.

DMA-based platforms are promising solutions for meeting the above-mentioned requirements. Two-dimensional molybdenum disulfide (MoS₂) nanosheets have good biocompatibility and bursting ability and protect loaded genes from enzymatic cleavage. Popova et al. proposed for the first time the entire process of culturing cells and then synthesizing cDNA in a nanoliter volume of droplets, demonstrating that the DMA platform can be used for the phenotypic analysis and processing of cells (Fig. 6A) [33]. Dendrimers are bio-interfaces with chemical stability and low cytotoxicity, and their DMA-based platforms can be easily grafted onto MoS₂ for biosensing [67]. The principle of a nanomaterial-based fluorescence resonance energy transfer (FRET) sensing assay is based on energy transfer from the donor to the acceptor [68]. Yang et al. developed an MoS₂ nanosheet-modified dendrimer DMA for the detection of HIV-I and HIV-2 [69]. They established an MoS₂ DMA FRET sensing platform by adsorbing a fluorescent dye-labeled oligonucleotide probe onto the surface of a hydrophilic spot coated with MoS₂ nanosheets as the donor and MoS₂ nanosheets as the acceptor for the FRET assay. In the presence of the target nucleic acid fragment, the probe is separated from the MoS₂ nanosheet surface, triggering the fluorescence signal to be

“on,” and conversely, in the absence of the target nucleic acid fragment, the fluorescence signal is off [37]. Their work validated that the platform has good sensitivity and specificity and has the advantages of low sample consumption and parallel detection without cross-contamination, which provides valuable insight into using this platform for various virus multiplex assays in the future. To bridge the performance gap of poor sensitivity in hybridization analysis-based nucleic acid detection and quantification techniques, Lo et al. developed a picoliter self-assembled DMA platform with greatly improved detection performance, detection limits as low as 570 copies and 50 am, a wide dynamic range (six orders of magnitude), and reduced reaction time (< 5 min) owing to the physically driven design and nanofeatures of the platform (Fig. 6B). Notably, this device is suitable for the detection and quantification of short nucleic acids and miRNAs for point-of-care applications and has various clinical applications in the rapid diagnosis of infectious diseases, cancer, brain injury, and other disorders [53].

Droplet microarray for high-throughput drug screening

Developing a new drug is a time-consuming and costly endeavor; the demand for new drugs is increasing, but the development of new drugs is decreasing. Between 1991 and 2000, 367 new drugs were developed in 21 major countries, while only 251 new drugs were developed in the following decade, and the cost of developing a new drug was reported to be more than \$2 billion [70, 71]. Thus, it is clear that the development of novel drugs is not only increasingly inefficient but also increasingly costly. Therefore, the innovative development of drug screening platforms is vital for improving the efficiency of drug screening. The development of

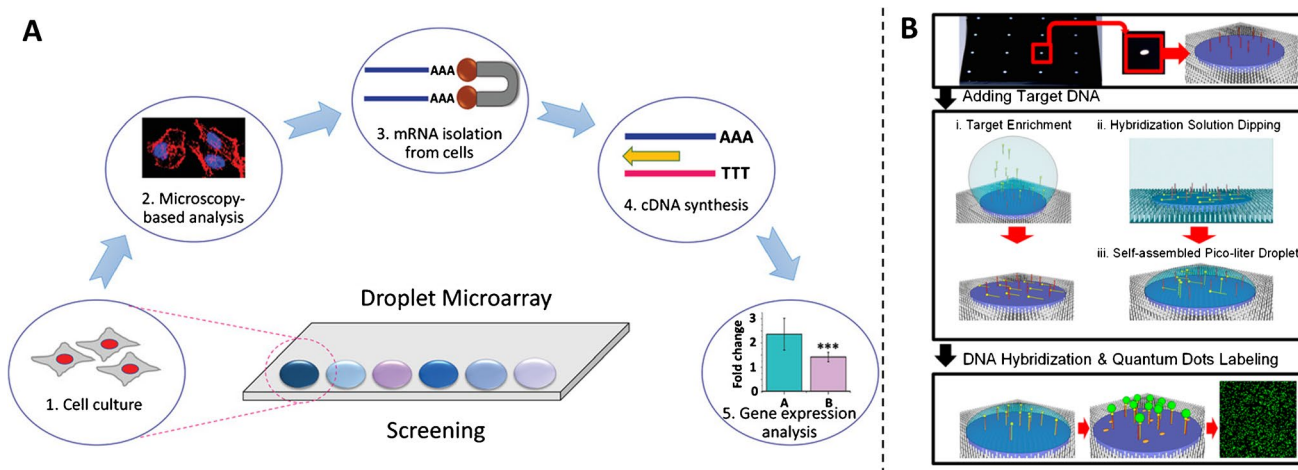


Fig. 6 Nucleic acid detection on DMA. **A** Workflow of “Cells-to-cDNA on Chip” [33]. Copyright 2015, American Chemical Society. **B** Picoliter droplet microarray for target nucleic acid hybridization assay workflow [53]. Copyright 2015, American Chemical Society

new drugs includes testing millions of chemical libraries for toxicity and metabolic function, relying on high-throughput cellular screening of large compound libraries [72, 73]. Animal models for drug screening are often costly and face ethical issues; therefore, *in vitro* cell-based drug screening has great potential. It has the advantage of reducing the cost and time of screening by reducing the number of animal experiments [74]. However, previous cell-based screens were typically performed in 96-well or 384-well plates with reaction volumes ranging from microliters to milliliters, requiring high cell count levels of reagent consumption, and, therefore, need to be further optimized.

Considering that the separation between chemistry (synthesis) and biology (analysis) in previous drug discovery processes has led to inefficiencies in the drug discovery process [75], Benz et al. developed a cell-based assay platform that combines chemical synthesis and biological readout on the same glass slide (chemBIOS). Small-scale chemical synthesis, characterization, and biological screening were performed uniformly in microarray form in microdroplets, a process that takes days rather than years (Fig. 7A). Using the chemBIOS platform, they synthesized lipidoid libraries and performed microarray preparation of lipoplex complexes and microarray cell screening to identify transfection reagents. The chemBIOS workflow for this experiment was based on a liquid-phase system, and in subsequent studies, Levkin et al. modified the DMA platform for use in solid-phase synthesis of small molecule assemblies and subsequent biological screening. Specifically, solid-phase synthesis at hydrophilic sites using photo-cleavable linkers allows the control of the final drug concentration in the separated individual droplet compartments by varying the irradiation time. They used peptide synthesis as a model reaction and tested the cell viability of HEK293T cells under UV irradiation, demonstrating the convenience and future potential of the chemBIOS approach based on solid-phase synthesis (Fig. 7B); solid-phase-based reactions also have additional advantages for avoiding cross-contamination [76].

The Ugi four-component reaction, widely used for rapid library creation, is a well-known multi-component reaction [77]. To achieve more complex combinatorial compound screening, Levkin et al. introduced a photocleavable linker as an anchor point for synthetic starting materials and then performed combinatorial solid-phase synthesis of a nanomolar library of more than 500 different compounds via the Ugi four-component reaction [3]. This was followed by surface and matrix-assisted laser desorption/ionization-time of flight MS analysis, which was performed on the same chip. Their experimental results demonstrated the added flexibility of the DMA platform in terms of time, space, and quantitative control to achieve miniaturized high-throughput screening [3]. Because the hygroscopicity of glycerol keeps the droplets constant, water and glycerol were combined in a

certain ratio (7:3). The resulting mixture was distributed by constructing a non-covalent array method for inkjet printers to form a biomolecular system of skin-upgraded DMA to optimize the rheology of and solve the evaporation problem in droplets, and the phase I metabolism was verified by photometric assay at the single-spot level. The inhibition of the enzyme system CYP3A4 was also verified using a photometric assay at the single-spot level. This study further validated the potential of DMA as a miniaturized high-throughput platform for drug screening [25].

The DMA platform is compatible not only with compound libraries but also with pathological cells such as cancer cells and bacteria that are subject to sexual drug screening. Levkin et al. established a platform for the rapid generation of nanoliter-sized droplet microarrays containing multidrug-resistant *Pseudomonas aeruginosa* to investigate the antibiotic resistance of *P. aeruginosa* 49 after evaluating its growth, validating that the DMA platform can rapidly form bacterial microarrays for high-throughput antibiotic resistance screening (Fig. 6C, D) [4]. Another study validated that the DMA platform can also be used for anti-cancer drug screening in patient-derived cancer cells (chronic lymphocytic leukemia) [35]. In summary, the DMA platform has great potential for drug screening applications because of its compatibility and ability to be used for any experiment. Users can even purchase ready-to-use DMA slides with custom pre-printed chemical libraries to enhance the experimental benefit.

Droplet microarrays for personalized and precision medicine

The evaluation of the efficacy and safety of individual drugs and the development of personalized strategies for the prevention and treatment of diseases are called precision medicine. The process may be done through the analysis of patient-specific blood samples, pathological biopsies, primary induced pluripotent stem cells, and personal health information to specify personalized health guidance.

Malignant tumors are characterized by high levels of intratumoral grade inter-tumoral heterogeneity. Therefore, treatment options can vary between patients with the same type of tumor in a timely manner, making it essential to perform drug sensitivity and resistance testing prior to treating individual tumor patients to determine the most appropriate treatment. However, in the past, the Drug Sensitivity and Resistance Test (DSRT) was usually performed in 96-, 384-, or 1536-well plates requiring 50–60,000 cells per well. This is not ideal in patients with rare cells of needle aspiration biopsy origin for analysis, and the number of cells is often insufficient for specimens to be used for both histopathological analysis and DSRT. Furthermore, the high cost of compounds and reagents makes the test expensive and financially

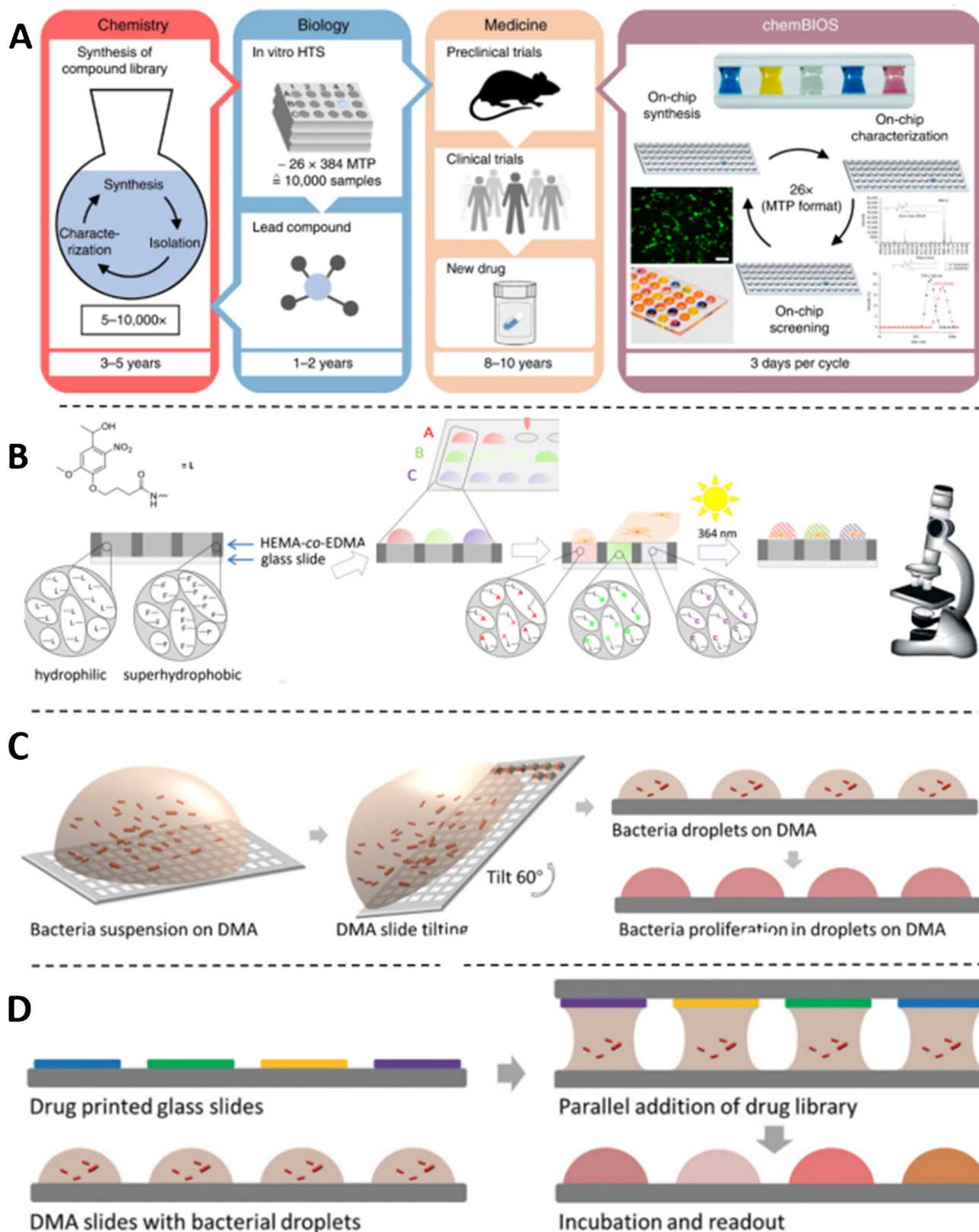


Fig. 7 A DMA enables chemical synthesis and biological analysis on the same platform, shortening drug development time [75]. Copyright, Nature Communications. B Schematic of chemBIOS device

with light-crackable connectors [76]. Copyright 2015, ELSEVIER. C Cultivation of bacteria on DMA; D antibiotic screening against bacteria by sandwich method [38]. Copyright 2020, Wiley Online Library

burdensome for early-onset patients. Popova et al. recently developed the application of miniaturized DSRT to primary patient-derived chronic lymphocytic leukemia cells by using nine anticancer compounds in pairs of cells from five different patients. The validation required only 100 cells per well compared to 384-well microplates, reducing cell consumption by 200-fold and reagent consumption by 300-fold. This demonstrates the significant potential of the DMA platform in the field of personalized and precision medicine [35].

Conclusion and future trends

This paper summarizes the applications of droplet microarrays, which rely on superhydrophilic-superhydrophobic surface properties to enable the culture of single cells, stem cells, and cell spheres in a single droplet and can be used to detect antibodies and small nucleic acids. Compared to traditional 96- and 384-well microplates, it greatly reduces the use of reagents, cells, compounds, and related consumables while improving experimental efficiency. Thus, it has great potential in basic research, drug development, precision medicine, and other biomedical fields.

Droplet microarrays are a powerful tool for high-throughput screening. First, in the fabrication of droplet microarrays, the physicochemical properties of droplets need to be controlled with very precise surface chemical parameters, including their wettability, stability, biocompatibility, and transparency. Therefore, there is a need for continuous improvement or development of materials for smart DMA. Second, as an open microreactor, each droplet is strictly independent of the other, but there are challenges for some reaction steps that need to observe the interaction between spheres, such as fusion and infiltration, and it is necessary to develop a programmable DMA platform that can be freely combined and easy to use. Droplet evaporation is also a notable issue that needs to be addressed. Currently [11], micro-droplets are prevented from evaporation by oil-coated droplets and by placing the droplet microarray in a humidity-controlled confined space. Other challenges include the need to diversify the methods of adding compounds to droplets in high throughput to meet different experimental needs. There is also a need for droplet microarrays to be compatible with a wider variety of readout methods for a more comprehensive analysis of microreactor results, currently most commonly used for microscopy, with recently developed methods such as electrochemical [15] and mass spectrometry [32], which are important breakthroughs in droplet microarrays.

Despite a number of challenges, this review clearly demonstrates the irreplaceable advantages of droplet microarrays for a variety of biological applications. These include single-cell analysis, cell sphere culture, stem cell research, drug screening, small-molecule detection, and personalized medicine.

Furthermore, droplet microarrays allow for the establishment of addressable microreactors in open nanoliter-to-microliter droplets, which greatly improves the efficiency and effectiveness of high-throughput screening experiments and contributes to the advancement of the discipline and industry.

Acknowledgements The authors thank the invitation of the editorial office and the valuable comments of all referees.

Author Contribution Conceptualization: L.S., S.L., X.L., X.H., and H.L.; writing—original draft writing: L.S., S.L., X.L., Z.L.; writing—review and editing: H.L., Z.L., L.W., Q.B., and X.D.; supervision: C.L., S.L. All authors have read and agreed to the published version of the manuscript.

Funding This study was supported by the Natural Science Foundation of Sichuan Provincial Department of Science and Technology (2022NSFSC1446), the Popularized Application Project of Sichuan Provincial Health Commission (No. chuan-gan-yan2023-214), the Central Universities Foundation of University of Electronic Science and Technology of China (No. ZYGX2019J109), and the National Natural Science Foundation of China (No. 62271184 and No. 81603018).

Data Availability Not applicable.

Declarations

Institutional Review Board Not applicable.

Informed Consent Not applicable.

Conflict of Interest The authors declare no competing interests.

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