ADVANCED MATERIALS INTERFACES
Open Access
www.advmatinterfaces.de

Droplet Microarrays for Miniaturized and High-Throughput Experiments: Progress and Prospectives

*D.D. Kartsev, Urrutia Gómez Joaquin E, Popova A. Anna, and Pavel A. Levkin**

Miniaturization in life sciences and chemical sciences offers substantial advantages to experimental workflows, such as increased throughput, reduced costs, and lower environmental impact. While microtiter plates are effective, further miniaturization is necessary to enhance efficiency and throughput. However, microtiter plates cannot be easily miniaturized to volumes below 5 µL, primarily because adhesive and capillary forces become stronger than the gravitational forces needed to confine the liquid within the wells. To overcome this, the droplet microarray (DMA) is developed, utilizing patterned adhesive regions on a liquid-repellent background to immobilize and confine sub-microliter droplets without physical barriers. This unique format enables novel applications such as droplet merging and parallel ultra-high-throughput manipulations. This review provides an overview of DMA's diverse applications and highlights the new experimental opportunities it offers, establishing it as a versatile tool for highly miniaturized, high-throughput biological and chemical experiments. The evolving requirements and future applications of the DMA approach are also discussed.

1. Introduction

In past years, a lot of effort has been put toward the development of novel miniaturized platforms. Miniaturization brings many advantages to both biological and chemical experiments, including higher throughputs, reduction of costs, and environmental burden. There are several basic requirements to effectively miniaturize test systems, namely: liquid confinement, parallelization, and automation. The majority of the high-throughput methods use wells or cavities for the physical confinement of liquids.[1,2] Although well-plates have proven effective in routine experimental workflows, and extensive infrastructure supporting these systems has been developed over the years, further miniaturization is essential to achieve the higher throughputs required to sustain the pace of modern research. Several studies on the direct miniaturization of well plates have been conducted.[3–5] However, in the further miniaturization of the test systems, gravity forces, which affect the liquid analytes, become too small in comparison to liquid-surface adhesive forces. Thus, a gravitational force of ≈9.8 µN acts on a 1 µL droplet. In contrast, the same droplet experiences adhesive forces between 100 and 200 µN[6,7] when in contact with a hydrophobic surface with an 800 µm diameter contact area—more than ten times stronger than gravity. Adhesive forces with a hydrophilic surface can be an order of magnitude stronger. Therefore, confining liquids with volumes below 5 µL using only physical solid borders becomes challenging, whereas confinement through spatially controlled adhesive and repellent regions becomes possible. This principle has been realized in open droplet microarrays, which confine and manipulate thousands of sub-microliter droplets on patterned surfaces for both biological and chemical experiments. In this review, we demonstrate how these changes in the test system's architecture affect its functionality and versatility.

To achieve adhesion patterning, regions of liquid adhesiveness (wettable) and repellency (non-wettable) need to be created. In our group, we introduced various types of such patterning techniques, mainly based on photochemical methods,[8–23] which resulted in the introduction of the concept of droplet microarrays (DMA).[24–52] DMA is a composite system consisting of a glass substrate and a thin coating with an applied wettability pattern. Individual biological or chemical experiments using DMA can be conducted in droplets ranging from 10 to 1000 nL, with the

D. Kartsev
International Institute "Solution Chemistry of Advanced Materials and Technologies" (SCAMT)
ITMO University
9 Lomonosova St., St. Petersburg 191002, Russia

U. G. Joaquin E, P. A. Anna, P. A. Levkin
Institute of Biological and Chemical Systems – Functional Molecular Systems (IBCS-FMS)
Karlsruhe Institute of Technology (KIT)
Hermann-von-Helmholtz-Platz 1, 76344 Eggenstein-Leopoldshafen, Germany
E-mail: levkin@kit.edu

U. G. Joaquin E
Institute of Automation and Applied Informatics (IAI)
Eggenstein-Leopoldshafen
Karlsruhe Institute of Technology (KIT)
76344 Karlsruhe, Germany

P. A. Anna, P. A. Levkin
Institute of Organic Chemistry (IOC)
Karlsruhe Institute of Technology (KIT)
Kaiserstraße 12, 76131 Karlsruhe, Germany

The ORCID identification number(s) for the author(s) of this article can be found under https://doi.org/10.1002/admi.202400905

DOI: 10.1002/admi.202400905

capacity to accommodate tens of thousands of droplets within the footprint of a standard microtiter plate.[10,46] (**Figure 1A,B**)

Though, the applicability of DMA is not only due to miniaturization. DMA is a flat platform and does not require physical barriers for holding liquids, representing a novel approach to high throughput miniaturized screenings. This simplification provides new possibilities for unique droplet manipulations, such as the use of discontinuous dewetting for experiment array application,[23,46,47,49] merging droplets on-demand,[43,45,48] the ability to perform cell culture using the hanging drop method,[47] and truly parallel droplet manipulations using the sandwiching method.[24] (Figure 1C,D) Furthermore, the modular structure of DMAs results in a wide range of test system parameters, variations of which can be utilized to fit the DMA platform for specific applications. For instance, variations in number, shape, and size of wettability compartments can be used in material microstructuring.[38,41,44] Adjusting the wettability contrast allows the formation of droplet arrays of different liquids.[40] Substrate functionality can be used to facilitate the operation of new on-chip readout systems.[39] Furthermore, chemical and physical functionalization of the wettable and non-wettable areas can be used for fine regulation of living cell adhesion[12,28,53] or differentiation of stem cells.[53,54] (Figure 1E) In this review, we examine various aspects of the DMA platform, developed primarily within our group, though not exclusively. This includes its design, patterning techniques, and the wide range of applications where DMA technology plays a pivotal role. We emphasize the potential of DMA as a versatile tool for future (ultra) high-throughput screenings and highly miniaturized chemical and biological experiments, precision medicine, and drug discovery. Additionally, we provide our perspective on the future evolution of the DMA platform, focusing on new experimental configurations and their potential applications.

2. Surface Patterning and Functionalization

A DMA is a composite device comprising multiple components, the collective functionality of which is determined by the manner in which these parts are assembled. It is noteworthy that both additive and synergistic contributions of these properties to the final application of the patterns are possible. This is why the combination of the substrate and the applied wettability pattern gives rise to a multitude of novel assays that allow for the exploration of new phenomena in various fields of science and engineering. This section will present the methods for creating DMAs, with a focus on the combinations of wettability patterning techniques and substrate properties.

In the case of highly rough surfaces, super wettability (e.g., superhydrophilicity) and super repellency (e.g., superhydrophobicity) can be achieved.[55] In this instance, the liquid is present on the non-wetted surface in a Cassie-Baxter state,[56] exhibiting apparent contact angles (both advancing and receding) of greater than 150 degrees. This can be achieved through the modification of the substrate, for example laser ablation, or the application of an additional coating layer, typically, nanoparticles.[57] Porous polymers[58] can be also employed to generate superhydrophobic/superhydrophilic wettability patterns. An alternative method for creating repellent interfaces is to achieve a liquid or quasi-liquid surface interface. The fundamental objective of these approaches is to achieve a combination of low surface energy and increased molecular mobility within the surface layer of the substrate. In the case of liquid interfaces, the porous substrate is impregnated with another immiscible, non-volatile, inert liquid of low surface tension, such as perfluoropolyether, thereby creating a surface layer that possesses liquid-like properties.[10,22,59] The formation of quasi-liquid interfaces is achieved through the grafting of mobile linear polymers onto the substrate surface.[23,39,60] The aforementioned approaches frequently result in repellency not only to water but also to liquids with lower surface tension, making them omniphobic and slippery. In this instance, the phenomenon is manifested in the form of low contact angle hysteresis values for different liquids.

In addition to wettability, substrate surface properties can significantly influence the functionality of a future DMA system. Substrates made of various materials can be used to create DMAs. The most common DMA is fabricated on a glass substrate, although using plastic,[23] paper,[18] and conductive materials such as ITO-coated substrates as a substrate is also possible. Cellu lose-based materials, such as paper,[61] are suitable for this field due to their low cost, biodegradability, and inherent porosity. The use of plastic as a substrate allows for the fabrication of flexible droplet microarrays. Applying wettability patterns to conductive surfaces, such as indium tin oxide-coated glass (ITO glass), significantly broadens the range of potential analytical readouts. This approach enables the analysis of organic compounds using techniques like Raman spectroscopy, IR spectroscopy, and MALDI-TOF-MS.[23,39] The integration of an omniphobic wettability pattern with advanced analytical methods offers a solution, for example, for on-chip organic synthesis combined with biological readouts.[39]

To create wettability patterns, the chosen substrate possessing micro-nano roughness coating undergoes the selective chemical functionalization. In general, the methods applied are based either on physical or chemical selective functionalization. Physical modification can include laser ablation of the material,[62] use of digital mirror device-based maskless lithography,[63,64] while chemical modifications may include material photolithography,[12] photografting[55] using various photochemical reactions, such as thiol-ene,[9,65] thiol-yne,[19] or tetrazole-thiol,[8] and material printing using inkjet[16] and physical stamping.[14]

Alternative methods for selective application of modifying reagents include physical stamping and inkjet printing of the functionalizing reagent. The resolution of inkjet printing depends on the substrate's wettability and roughness. However, it typically limits modifications to circular shapes. A key advantage is the reduced consumption of reagents.[14]

Beyond chemical factors, pattern design includes control over the shape, size, number, and density of wettability regions. This flexibility enables a wide range of applications, combining open microfluidics with droplet arrays and lab-on-a-chip technologies. For example, controlling microdroplet shapes has been used to create hydrogel microparticles with defined forms[41] and metal-organic framework microsheets shaped by the geometry of water droplets.[38,44] Recently, a method for the direct, selective deposition of porous polymethacrylate films using omniphobic-omniphilic wettability patterns to control adhesion was developed. The patterns were produced by using an omniphobic

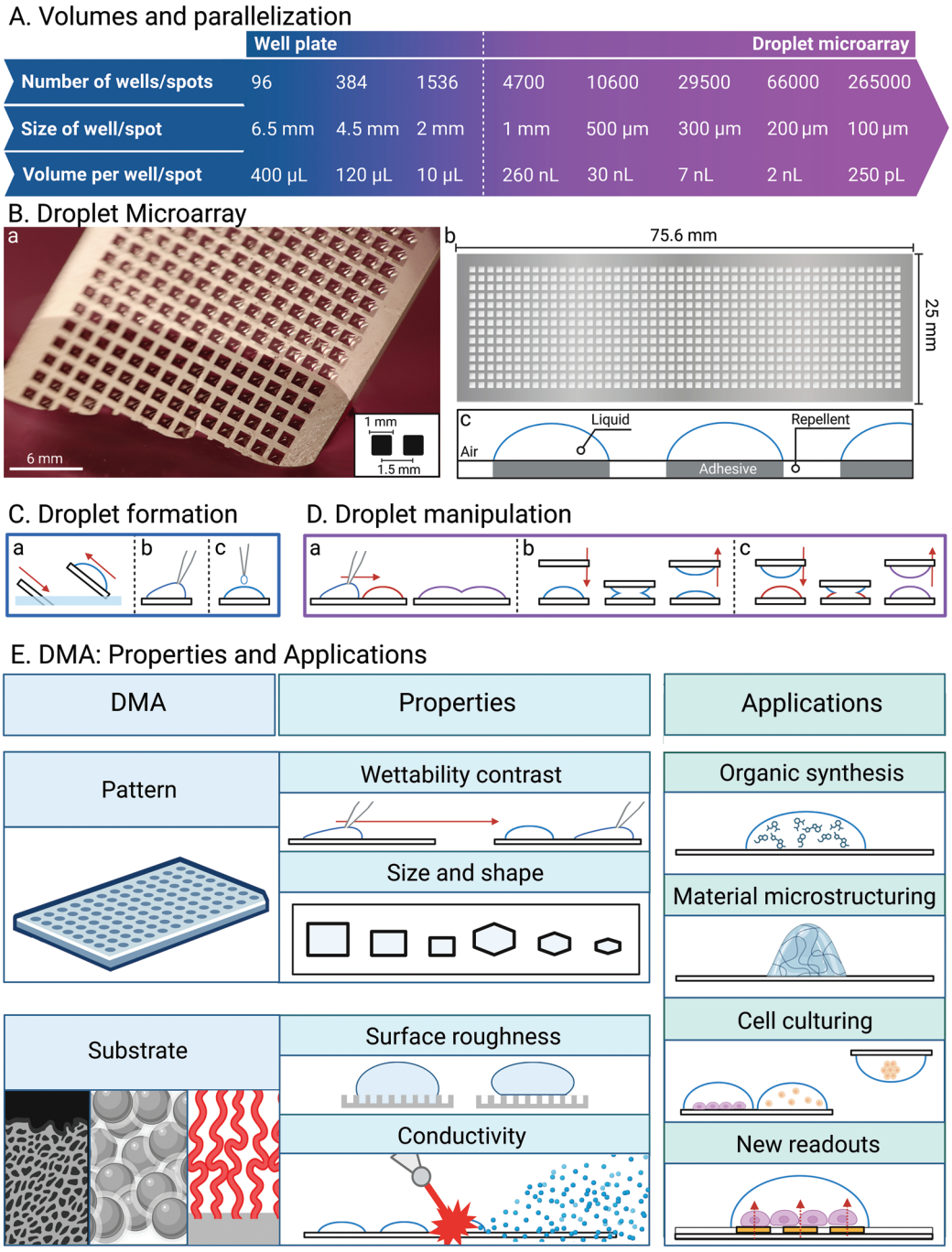

Figure 1. Overview of the Droplet Microarray (DMA) A.) Comparative illustration showing the level of miniaturization achievable with the DMA, both in terms of volume and the number of experimental points, compared to microtiter plates. The measurements and volumes are estimated values. B.a.) Image of a DMA with 672 spots immersed in cell culture medium. B.b.) illustration depicting the dimensions of a standard microscope slide commonly used as a substrate for the DMA. B.c.) Schematic showing droplets formed on the DMA, confined to superhydrophilic spots by the surrounding superhydrophobic topography. C.) Illustration depicting three different methods for droplet formation on the DMA: a.) Spontaneous droplet formation induced by discontinuous dewetting upon submersion of the DMA in liquid. b.) Rolling drop method, where daughter droplets are generated from a mother droplet as it rolls across the DMA. c.) Manual or automated droplet printing directly onto the spots using pipetting or liquid printers. D.) Illustrations depicting three unique droplet manipulations that can be performed on DMA: a.) Merging of neighboring droplets on a one DMA b.) Transition of droplets from one DMA to another using the sandwiching method c.) Merging of droplets positioned on different DMAs by using the sandwiching method E.) The table illustrates key properties of the DMA, such as the on-demand creation of spots with different shapes and sizes tailored to specific applications. Variations in the substrate's topography or conductivity enable a wide range of applications and analyses, including, but not limited to, organic synthesis, hydrogel formation, and cell culture, followed by analysis using microscopy, spectroscopy, or mass spectrometry, among others. Images reproduced with permission from the publisher.

photoresist based on the selective photoacid polymerization of octa(3-glycidyloxypropyl) polyhedral oligomeric silsesquioxane modified with mono-aminopropyl-terminated polydimethylsiloxane (GPOSS-PDMS)[12] Selective adhesion to GPOSS-PDMS patterns allowed polymethacrylate microstructuring without droplet array formation. Since there was no need to form droplets to create microstructures, the problem of droplet evaporation was eliminated. As a result, wettable areas down to 100 µm could be utilized. The shape and size of the polymethacrylate structures were defined by the wettability pattern design. Additionally, films with varying surface chemistry and porosity were achieved by using different precursor solutions.[15]

3. New Functionalities Enabled Through the Use of Wettability Patterns

A distinct liquid confinement mechanism in DMAs, compared to microtiter plates, expands the range of possible droplet manipulations. A key feature is the effect of discontinuous dewetting (**Figure 2A**), which enables the rapid, single-step formation of thousands of nanoliter droplets on a DMA surface.[22,66] When a DMA slide is immersed in liquid or a droplet is rolled across its surface, the liquid adheres to adhesive spots and dewets from repellent regions, forming nanoliter droplets in the hydrophilic or omniphilic regions. This technique is particularly valuable for generating thousands of confined compartments containing cells, bacteria, microorganisms, particles, or droplets with dispersed or dissolved molecules for high-throughput parallel experiments.[27,28,67,68](Figure 2A.i–iv) Discontinuous dewetting also facilitates the material microstructuring on DMA. Thus, Neto et al. demonstrated the fabrication of freestanding sheet-like poly(ϵ-caprolactone) (PCL) microparticles encompassing a vast array of geometries with high precision.[61] The sacrificial layer of poly(acrylic acid) (PAA) was applied by discontinuous dewetting and solvent evaporation of the PAA alcohol-water solution. Afterward, the same operation was repeated for PCL dichloromethane solution. Due to the pH responsiveness-driven molecular reconfiguration and further dissolution of PAA, NaOH was effective on promoting the etching of the sacrificial polymer layer, leading to the release of PCL microparticles from the glass substrate into the solution. (Figure 2A.a–d)

Another interesting feature of the DMA platform is the ability to merge adjacent droplets on demand, thanks to the absence of physical solid barriers between them. This can be achieved by dispensing slightly more liquid into neighboring droplets, causing them to contact and merge into a single, larger droplet, allowing for unique sample interactions. This capability has been exploited to create complex 3D cell assembloids by merging droplets containing individual spheroids.[48] Once merged, these spheroids are cultured using the hanging drop method, where they fuse to form complex multi-spheroid 3D architectures (Figure 2B.i–iv). This feature also supports the co-culture of 2D and 3D cell models within individual nanoliter droplets, as well as the formation of hetero-spheroid assemblies.[69] Moreover, Wiedmann et al. employed the droplet merging technique to perform high-throughput liquid-liquid extractions at the scale of 200 nL droplets, an essential method for purifying complex mixtures of synthesized molecules on DMA.[43] (Figure 2B.a–e)

Another key advantage of the DMA platform over microtiter plates is the small droplet volumes and the dominance of adhesive forces over gravitational forces, which ensures high droplet stability regardless of orientation. Even when the DMA is inverted, droplets remain securely attached to the surface. This feature enables several unique applications. First of all, this method allows the formation of arrays of cell spheroids by culturing cells in the inverted orientation, which can be used for example for drug screenings.[70] This advantage also facilitates highly parallel, high-throughput droplet manipulations using the sandwiching technique, where two DMAs are aligned and brought into contact, facilitating the merging of droplets from the primary array with those on the secondary array (Figure 2C). The previously discussed simultaneous and highly parallel manipulation techniques have demonstrated multiple applications. For instance, the sandwiching method has enabled the development of new on-chip analytical methods, such as using this setup to trap droplets between two DMAs, allowing for UV–vis spectroscopic measurements directly on-chip.[39](Figure 2C.i,ii) This technique has also been applied for cell culture management on the DMA, including non-destructive sampling, where part of the cell culture volume is extracted and analyzed from a copy created in another DMA by removing the medium through the sandwiching method. Furthermore, it has demonstrated the ability to refresh cell culture media by first removing the old media from thousands of droplets in parallel, and then introducing fresh media using a liquid dispenser, thereby extending the viability of the cell cultures. Furthermore, this technique has been employed to transfer thousands of spheroids in seconds from one DMA to another by placing the slides in sandwiching mode. Liquid placed within the wettable areas of the patterns is stably confined and will not separate from the surface even when the DMA is inverted. However, objects placed within the liquid droplets may move within the medium in response to gravitational forces when the DMA is tilted. Thus the spheroids settle by gravity from the upper slide to the lower one.[71](Figure 2C.a–e) Zhao et al. used the sandwiching technique to perform the simultaneous addition of drugs in thousands of individual droplets. In the experimental set used, each droplet followed its own exposure protocol. As a result, this approach facilitated programmed, concurrent, and uninterrupted drug treatments for a vast number of droplets during high-throughput screening, all managed by computer software.[64] (**Figure 3A**) Sun et al. utilized printing of porous polymers on transparent substrates to confine and merge multiple immiscible liquid layers to form droplet-based microreactors with real-time spectroscopic monitoring. This strategy enabled easy customization of 2D chips for chemical processes, successfully demonstrating microextraction and synthesis within stable multiphase liquid layers in droplets. By integrating an automatic liquid dispenser and microplate reader, the platform achieved automated liquid addition, simultaneous reaction initiation, accelerated reactions through shaking, and real-time monitoring, advancing toward fully automated synthesis.[72](Figure 3B)

Despite significant progress in developing complex protocols on DMAs—enabled by the integration of liquid dispensers for precise droplet formation and compatibility with optical readouts—a challenge remains in interfacing DMAs with conventional methods such as HPLC-MS, NMR, and other off-chip analytics. This requires the transfer of nanoliter droplets

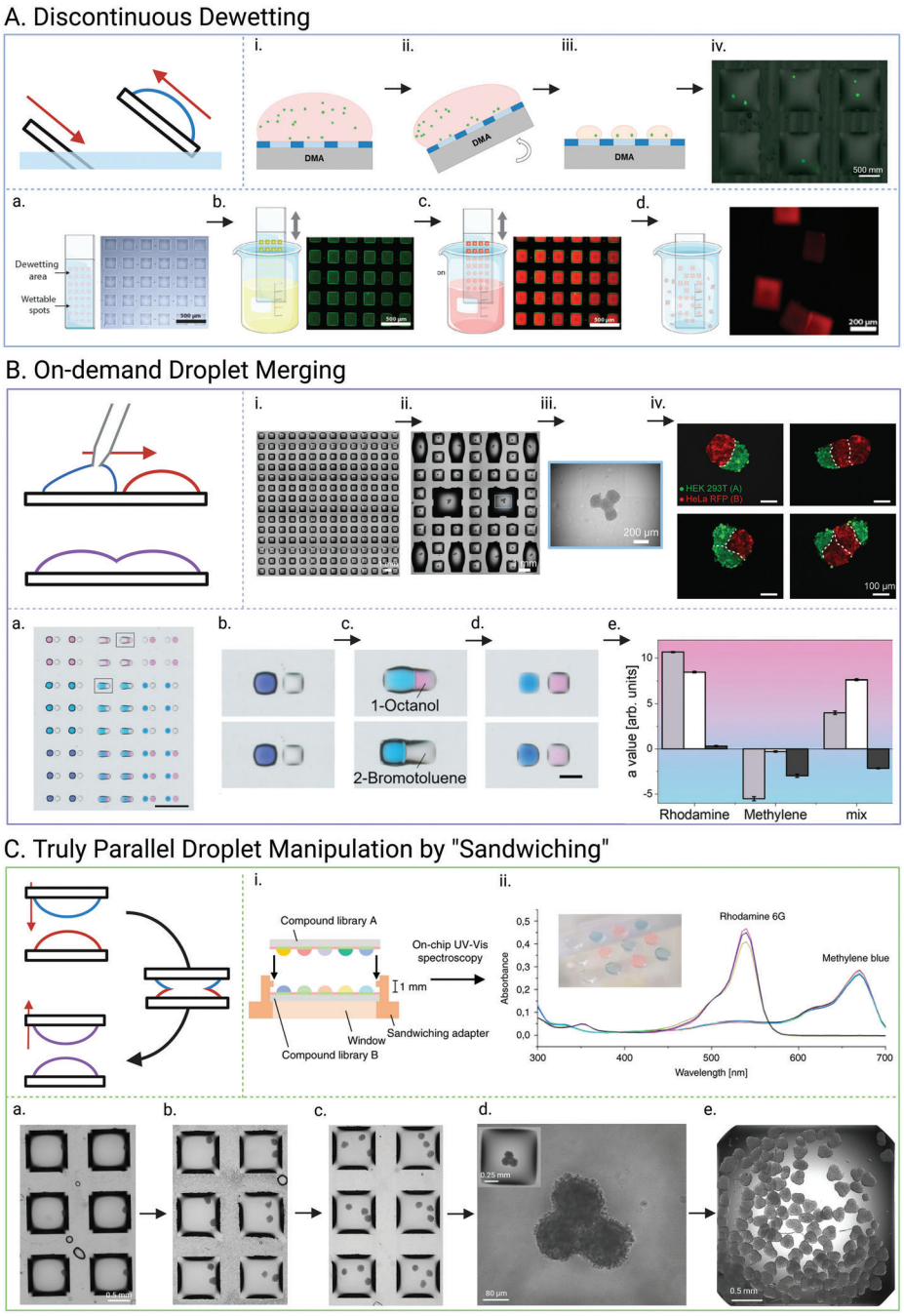

Figure 2. Droplet Microarray applications and analysis. A.) Discontinuous Dewetting. A.i–iv) Schematic showing the workflow for the formation of single cells on the DMA using the manual finite dilution method through discontinuous dewetting. This study examined the viability and proliferation of single cells on the DMA.[27] A.a–d) Illustration showing the experimental procedure for the fabrication of quasi-2D poly(ϵ-caprolactone) (PCL) microparticles, achieved by submerging the DMA in various solutions. Fluorescence images reveal the final formation of microparticles.[73] B.) On-demand Droplet Merging. B.i–iv) Images demonstrating the use of droplet merging to fuse neighboring spheroids of different cell types, each created independently in individual spots on the DMA. These droplets were merged to form double and triple spheroids with different geometries, guided by the direction of the merging process. Fluorescence microscopy images show the resulting hetero-spheroid architectures formed from different cell lines.[48] B.a–e) Image representing the use of on-demand droplet merging to combine two neighboring droplets containing different compounds to perform a nanoliter scale parallel liquid-liquid extraction.[43] C.) Truly Parallel Droplet Manipulation by Sandwiching. C.i–ii) Schematic depicting the use of sandwiching to perform an on-chip UV–vis spectroscopy, the accompanying graph shows the on-chip measured UV–vis spectra of rhodamine and methylene blue.[39] C.a–e) Images showing the transfer of spheroids from one DMA to another, placing them in individual spots in a fast, parallel manner using sandwiching. These spheroids were then merged and cultured in each individual spot, in contrast to the neighboring spheroid merging method, resulting in a complete array of multiple spheroids. As a final result, the merged spheroids were harvested and collected into a vessel for subsequent use and experimentation.[71] Images reproduced with permission from the publisher.

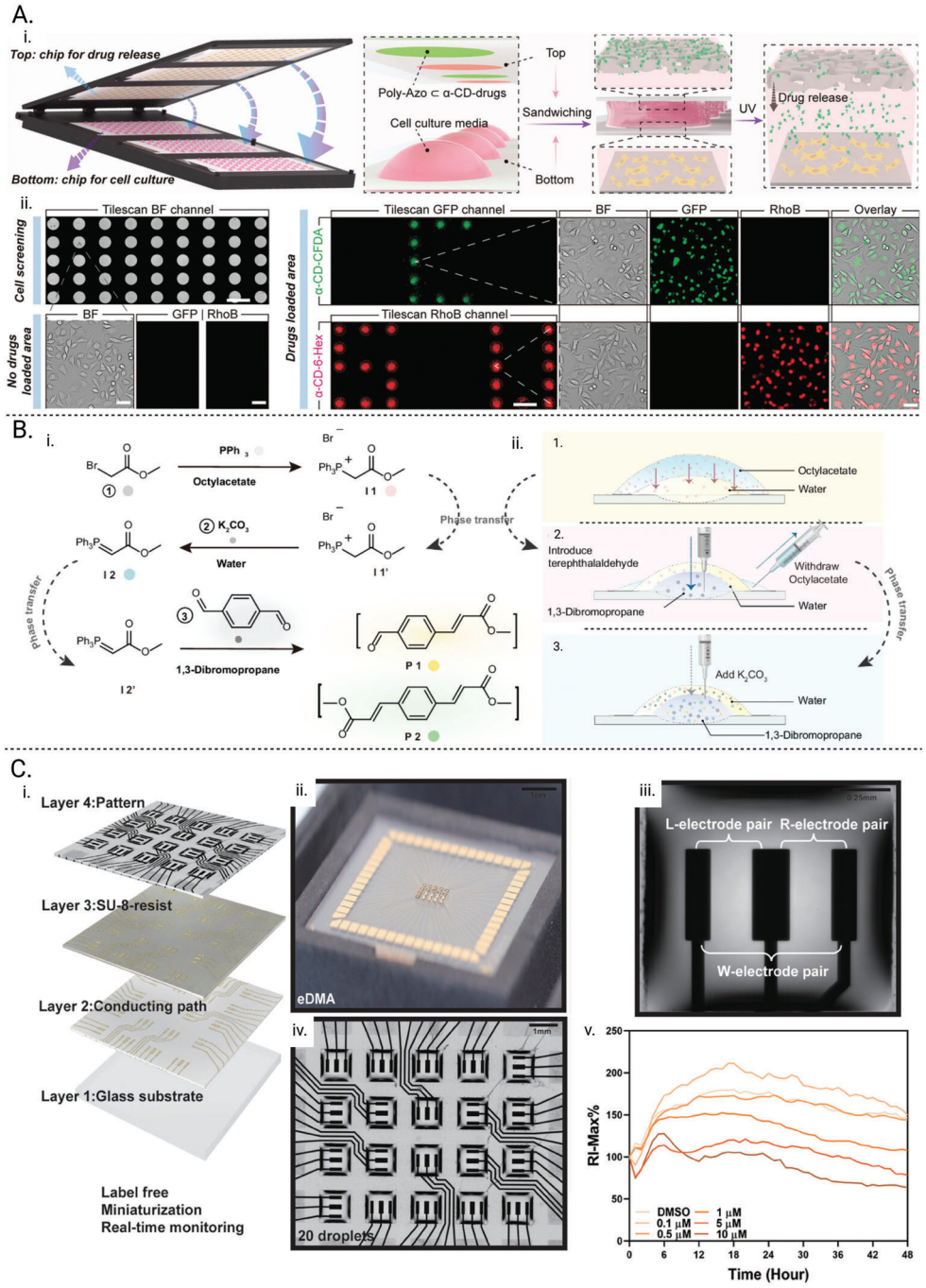

Figure 3. New methods and applications on Droplet Microarrays. A. i) Schematic showing the procedure for UV light-induced drug release in droplets containing cells using the sandwich method published by Zhao et al.[64] A. ii) Fluorescence microscopy images of the droplet array after 122 h following the parallel addition of drugs via UV light inductional. B. i, ii) Schematic from the Sun et al. study illustrating the chemical synthesis of dimethyl 1,4-phenylenediacrylate carried out in a three-layer-stacked droplet microreactor on a chip.[72] C.) An example of impedance spectroscopy analysis on-chip, published by Zhou et al. In images i–iv, the embedded electrodes in the substrate are shown, allowing droplets containing cells to be positioned on the DMA spots for analysis.The graphic v display impedance readings over 48 h, studying the effect of different concentrations of Doxorubicin on cell cultures.[75] Images reproduced with permission from the publisher.

to external equipment. To address this, the Automated Nanoliter Droplet Selection and Collection Device (ANDeS) was recently introduced.[74] This automated droplet manipulation device collects samples from droplets into a fused silica capillary. These capillaries serve both as containers, aiding in droplet handling and preventing evaporation, and as vessels for further processing and transport for subsequent analysis. Evaporation is significantly slowed. After collection, the ends of the capillaries are sealed with silicone septa, preventing the samples from coming into contact with the external environment. Evaporation is reduced from minutes to days by reducing the surface area of the liquid-air interface. In addition, the vapor concentration is saturated in the air pockets left between the samples due to the sealing.

The future of DMAs lies not only in improving their compatibility with external instruments but also in advancing the platform's capabilities. Thus, integrating technologies such as embedded electrodes directly into DMAs could enable electrochemical on-chip analysis of droplet content. A notable example is the Electrode Droplet Microarray (eDMA), a bioelectrical monitoring system for real-time, label-free cellular viability assessment using impedance spectroscopy.[75] In this system, microelectrodes fabricated on a silicon substrate are coated with nanoparticles and patterned via photolithography to create hydrophilic-superhydrophobic regions that position nanodroplets over individually addressable electrodes printed on a glass substrate, connecting the DMA to an impedance analyzer for individual spot readings (Figure 3C). This approach effectively monitored the cytotoxic effects of an anticancer drug in real time over 48 h in 200 nL droplets, without the need for additional staining of the cells.[75] In addition to enabling electrochemical analysis, addressable electrodes could allow for selective droplet manipulation. For example, integrating thermoelectric modules for localized heating, could support applications like on-chip chemical reactions, sample preparation or PCR. The incorporation of microtechnology into DMA substrates offers enhanced droplet handling and on-chip analysis, facilitating the transition from nanoliter to picoliter droplets in even denser arrays.

Another unique feature of DMAs is their glass substrate, compared to the plastic used in microtiter plates. This allows DMAs to withstand higher temperatures, enabling processes like nucleic acid conversion directly on the array, without the need to transfer cells from culture vessels to specialized tubes. For instance, Chakraborty et al. pioneered a workflow for isolating mRNA from living cells and converting it to cDNA on the same DMA, a process requiring heating up to 82 °C.[76] Recently, Faraj et al. developed an improved protocol for drug-induced differential gene expression analysis on a low number of patient-derived chronic lymphocytic leukemia (CLL) cells.[77] Such techniques enable a molecular-level analysis of drug effects on cells in nanoliter droplet, all on a single DMA chip.

4. DMAs for High Throughout Biological and Chemical Applications

DMAs are widely recognized for their utility in high-throughput screening applications across various model systems, including eukaryotic cells,[24,28,70,78] bacteria,[31,51,68,79] (**Figure 4A**), and even small organisms.[35] In a notable study, Cui et al. presented a screening workflow that tested over 2000 FDA-approved drugs on patient-derived IDH1 mutant glioma tumorspheres using a single-tumorsphere droplet array. This approach identified 20 promising drug candidates with potential for repurposing in the treatment of glioma patients with this tumor profile.[70] Similarly, Lei et al. conducted a screening of a library of more than 2000 novel compounds against carbapenem-resistant *Klebsiella pneumoniae* and methicillin resistant *Staphylococcus aureus*, utilizing DMAs and a colorimetric viability read-out to identify potential drug candidates for treating multidrug-resistant bacteria.[79]

Beyond high-throughput applications, DMAs are particularly well-suited for situations where cell availability is limited, such as in functional precision oncology. Here, biopsy-derived cells are exposed to a range of clinically relevant drugs to identify tailored therapies for individual patients.[37,80,81] Additionally, DMAs are compatible with the creation of 3D scaffolds; for example, hydrophilic spots on DMAs can be used to print hydrogels, including detachable, functionalized or photodegradable hydrogels.[41,42,82] Furthermore, DMAs have been adapted for small-animal models. Popova et al. demonstrated the incubation of zebrafish embryos in 5 µL droplets for 24 h, followed by exposure to cytotoxic agents and fluorescently labeled peptoids.[35](Figure 4B)In summary, DMAs have proven to be a versatile platform for high-throughput miniaturized screenings across a wide range of biological models.

The miniaturization and increased throughput of chemical synthesis are essential for enhancing efficiency and enabling integration with biological screenings on a single platform. This integration is crucial for accelerating drug discovery, shortening experiment times, and reducing the environmental impact of these processes. Traditionally, chemical synthesis is done in large volumes, one compound at a time, before testing on cells. In the ChemBIOS concept, combinatorial libraries of small molecules can be synthesized in high throughput in nanoliter droplets on DMAs, either using solid-phase or liquid-phase formats, and directly tested on proteins, eukaryotic cells or bacteria.[39,83,84] (Figure 4C) For instance, Seifermann et al. utilized the combinatorial solid-phase synthesis approach to generate a library of 132 PROTAC-like molecules via solid-phase Ugi reaction, and tested for anticancer activity in HT-29 colorectal adenocarcinoma cells.[83] ChemBIOS holds significant, yet not fully discovered, potential to transform chemical synthesis and compound testing, and therefore – the field of drug discovery.

These applications, combined with DMA's novel features—such as extreme miniaturization, ultra high-throughput capabilities, parallel manipulations via sandwiching, droplet merging, and cell spheroid formation using the hanging drop method—offer numerous new possibilities. By leveraging miniaturization and high throughput, DMAs can help reduce the environmental impact of large-scale experiments while simultaneously lowering costs and accelerating research.

5. Conclusion

In conclusion, Droplet Microarrays (DMAs), introduced by our research group, present a versatile solution to many challenges in high-throughput screening (HTS) and in miniaturization of both biological and chemical experiments. By merging the advantages of microtiter plates, droplet microfluidics, and open

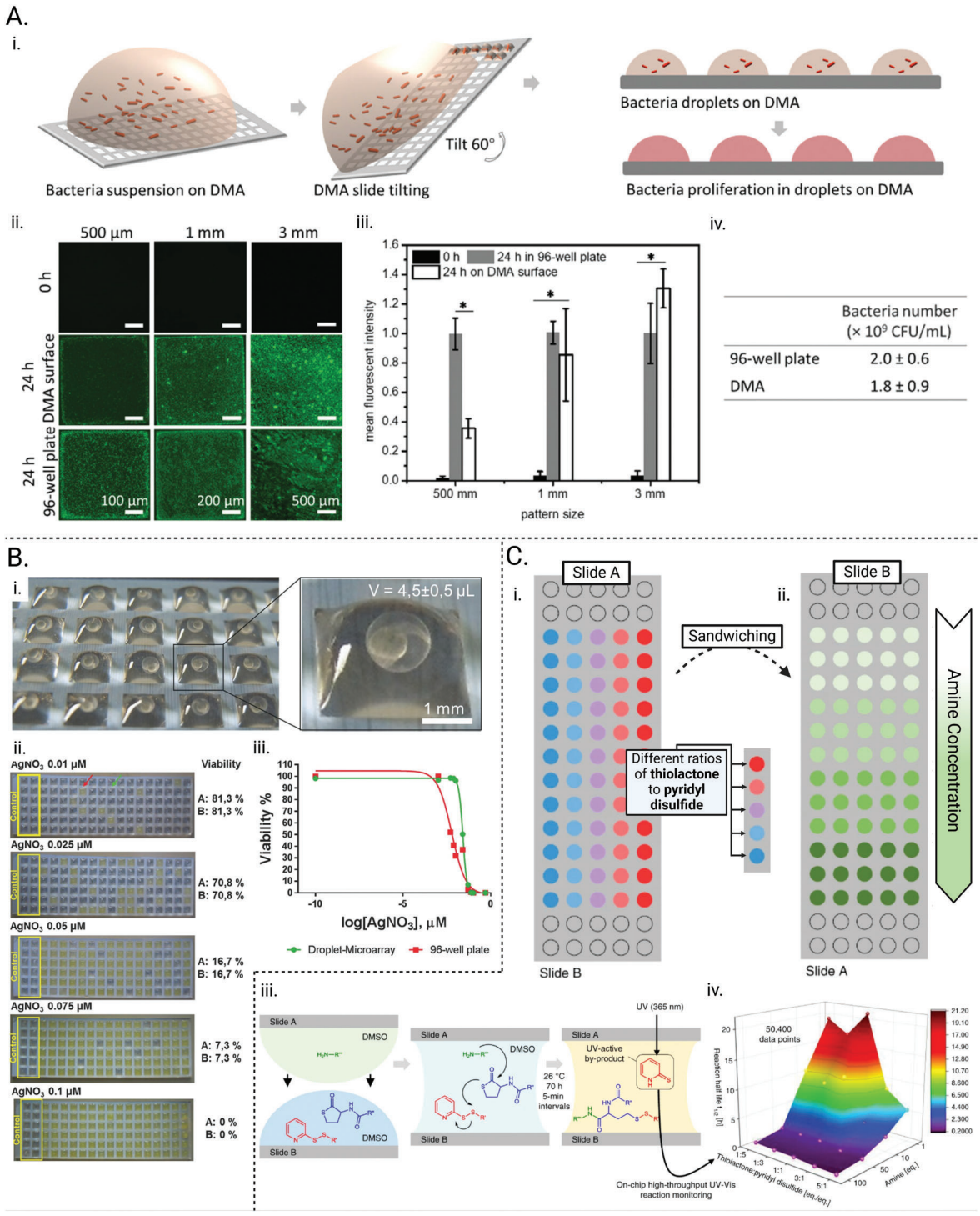

Figure 4. High-throughput analysis on DMA. A.i) Schematic of the discontinuous dewetting method used for seeding bacteria on the DMA. A. i–iv) Results showing the growth of *P. aeruginosa* GFP incubated for 24 h on a DMA compared to its growth in 96-well plates, for subsequent use in the screening of antimicrobial compounds.[68] B. i) An image of a DMA containing zebrafish embryos, spread by the effect of discontinuous dewetting, 24 h post-fertilization. Toxicological screening of zebrafish embryos conducted on a DMA. The images show DMAs with zebrafish embryos exposed to $AgNO_3$ for 24 h. The graphs display the number of hints provided by the image in each case, illustrating the effect of $AgNO_3$ on zebrafish embryo viability. The graph compares screening results conducted on DMA versus a 96-well plate format.[35] C.) On-chip synthesis by sandwiching of lipidoid with UV–vis readout. i, ii) Represent the array layout of each DMA slide: Slide A contains different ratios of thiolactone to pyridyl disulfide, while Slide B contains various concentrations of amine. The reactions between compounds are conducted simultaneously by sandwiching both slides, as shown in iii). iv) The result of this synthesis is demonstrated through on-chip UV–vis analysis, displayed as a 3D plot of the reaction half-life, confirming the feasibility of this method.[39] Images reproduced with permission from the publisher.

microfluidics, DMAs enable the formation and manipulation of thousands of nanoliter-scale droplets through their open, planar, wall-less design and extreme wettability contrast. This allows for both chemical synthesis and biological assays in a highly miniaturized format, while maintaining compatibility with analytical techniques like mass spectrometry and optical spectroscopy. The precision of droplet placement using discontinuous dewetting and the ability to perform parallel droplet manipulations further enhance the platform's efficiency. These features make DMAs a useful tool for ultra high-throughput assays, particularly in drug discovery, by enabling complex, multi-step workflows with reduced experimental volumes. Moreover, DMAs address key benefits of miniaturization, such as lowering reagent consumption, reducing costs, and minimizing environmental impact. They have proven particularly advantageous in applications like drug screening, where limited sample availability, such as cancer cell biopsies, makes DMAs a superior alternative to traditional well plates in the field of functional precision medicine. To fully realize the potential of this technology, ongoing research must focus on expanding its capabilities and developing new analytical and automation methods that integrate seamlessly into conventional laboratory workflows.

Acknowledgements

P.A.L. thanks the Deutsche Forschungsgemeinschaft (DFG) (Heisenberg-professur project number 406232485, LE 2936/9-1) for the financial support. J.E.U.G. thanks the German Academic Exchange Service (DAAD) (Research Grants – Doctoral Programmes in Germany, 57588370) for the financial support. The work of Kartsev, D.D. was supported by the national project "Science and Universities", project No. FSER-2022-0008.

Conflict of Interest

The authors declare no conflict of interest.

Keywords

cell microarrays, droplet microarray, experiment miniaturization, high throughput compound screening, wettability patterns

Received: November 12, 2024
Revised: December 9, 2024
Published online:

[1] M. Zhang, Y. Zhang, Y. Wang, W. Lv, Y. Zhang, *Sci. Rep.* **2019**, *9*, 6023.
[2] F. Madoux, A. Tanner, M. Vessels, L. Willetts, S. Hou, L. Scampavia, T. P. Spicer, *SLAS Discov* **2017**, *22*, 516.
[3] P. C. Chen, Y. Y. Huang, J. L. Juang, *Lab Chip* **2011**, *11*, 3619.
[4] S. Lindström, M. Hammond, H. Brismar, H. Andersson-Svahn, A. Ahmadian, *Lab Chip* **2009**, *9*, 3465.
[5] S. Lindström, R. Larsson, H. A. Svahn, *Electrophoresis* **2008**, *29*, 1219.
[6] V. Liimatainen, M. Vuckovac, V. Jokinen, V. Sariola, M. J. Hokkanen, Q. Zhou, R. H. A. Ras, *Nat. Commun.* **2017**, *8*, 1798.
[7] B. Samuel, H. Zhao, K. Y. Law, *J. Phys. Chem. C* **2011**, *115*, 14852.
[8] W. Feng, L. Li, C. Yang, A. Welle, O. Trapp, P. A. Levkin, *Angew. Chem., Int. Ed.* **2015**, *54*, 8732.
[9] J. Li, L. Li, X. Du, W. Feng, A. Welle, O. Trapp, M. Grunze, M. Hirtz, P. A. Levkin, *Nano Lett.* **2015**, *15*, 675.
[10] E. Ueda, P. A. Levkin, *Adv. Healthcare Mater.* **2013**, *2*, 1425.
[11] P. Auad, E. Ueda, P. A. Levkin, *ACS Appl. Mater. Interfaces* **2013**, *5*, 8053.
[12] D. D. Kartsev, A. Y. Prilepskii, I. M. Lukyanov, E. G. Sharapenkov, A. V. Klaving, A. Goltaev, A. Mozharov, L. Dvoretckaia, I. Mukhin, P. A. Levkin, *Adv. Mater. Interfaces* **2023**, *10*, 2300156.
[13] L. Li, J. Li, X. Du, A. Welle, M. Grunze, O. Trapp, P. A. Levkin, *Angew. Chem., Int. Ed.* **2014**, *53*, 3835.
[14] M. Hirtz, W. Feng, H. Fuchs, P. A. Levkin, *Adv. Mater. Interfaces* **2016**, *3*, 1500469.
[15] D. D. Kartsev, I. M. Lukianov, E. G. Sharapenkov, A. Y. Prilepskii, P. A. Levkin, *Adv. Mater. Interfaces* **2024**, 2400569.
[16] J. S. Li, E. Ueda, A. Nallapaneni, L. X. Li, P. A. Levkin, *Langmuir* **2012**, *28*, 8286.
[17] J. Guo, W. Fang, A. Welle, W. Feng, I. Filpponen, O. J. Rojas, P. A. Levkin, *ACS Appl. Mater. Interfaces* **2016**, *8*, 34115.
[18] J. Guo, I. Filpponen, L. S. Johansson, S. Heiβler, L. Li, P. Levkin, O. J. Rojas, *Cellulose* **2018**, *25*, 367.
[19] W. Feng, L. Li, E. Ueda, J. Li, S. Heißler, A. Welle, O. Trapp, P. A. Levkin, *Adv. Mater. Interfaces* **2014**, *1*, 1400269.
[20] L. Li, W. Feng, A. Welle, P. A. Levkin, *Angew. Chem., Int. Ed.* **2016**, *55*, 13765.
[21] X. Du, J. Li, A. Welle, L. Li, W. Feng, P. A. Levkin, *Adv. Mater.* **2015**, *27*, 4997.
[22] D. Paulssen, W. Feng, I. Pini, P. A. Levkin, *Adv. Mater. Interfaces* **2018**, *5*, 1800852.
[23] W. Feng, L. Li, X. Du, A. Welle, P. A. Levkin, *Adv. Mater.* **2016**, *28*, 3202.
[24] A. A. Popova, S. M. Schillo, K. Demir, E. Ueda, A. Nesterov-Mueller, P. A. Levkin, *Adv. Mater.* **2015**, *27*, 5217.
[25] F. L. Geyer, E. Ueda, U. Liebel, N. Grau, P. A. Levkin, *Angew. Chem., Int. Ed.* **2011**, *50*, 8424.
[26] E. Ueda, W. Feng, P. A. Levkin, *Adv. Healthcare Mater.* **2016**, *5*, 2646.
[27] G. Jogia, T. Tronser, A. Popova, P. Levkin, *Microarrays* **2016**, *5*, 28.
[28] T. Tronser, A. A. Popova, M. Jaggy, M. Bastmeyer, P. A. Levkin, *Adv. Healthcare Mater.* **2017**, *6*, 1700622.
[29] M. Brehm, S. Heissler, S. Afonin, P. A. Levkin, *Small* **2020**, *16*, 1905971.
[30] T. Tronser, A. A. Popova, P. A. Levkin, *Curr. Opin. Biotechnol.* **2017**, *46*, 141.
[31] W. Lei, P. Krolla, T. Schwartz, P. A. Levkin, *Small* **2020**, *16*, 2004575.
[32] W. Lei, K. Demir, J. Overhage, M. Grunze, T. Schwartz, P. A. Levkin, *Adv. Biosyst.* **2020**, *4*, 2000073.
[33] T. Tronser, K. Demir, M. Reischl, M. Bastmeyer, P. A. Levkin, *Lab Chip* **2018**, *18*, 2257.
[34] G. Oudeng, M. Benz, A. A. Popova, Y. Zhang, C. Yi, P. A. Levkin, M. Yang, *ACS Appl. Mater. Interfaces* **2020**, *12*, 55614.
[35] A. A. Popova, D. Marcato, R. Peravali, I. Wehl, U. Schepers, P. A. Levkin, *Adv. Funct. Mater.* **2018**, *28*, 1703486.
[36] A. N. Efremov, E. Stanganello, A. Welle, S. Scholpp, P. A. Levkin, *Biomaterials* **2013**, *34*, 1757.
[37] A. A. Popova, S. Dietrich, W. Huber, M. Reischl, R. Peravali, P. A. Levkin, *SLAS Technol* **2021**, *26*, 274.
[38] A. Laromaine, T. Tronser, I. Pini, S. Parets, P. A. Levkin, A. Roig, *Soft Matter* **2018**, *14*, 3955.
[39] M. Benz, A. Asperger, M. Hamester, A. Welle, S. Heissler, P. A. Levkin, *Nat. Commun.* **2020**, *11*, 5391.
[40] M. Benz, M. R. Molla, A. Böser, A. Rosenfeld, P. A. Levkin, *Nat. Commun.* **2019**, *10*, 2879.
[41] A. I. Neto, K. Demir, A. A. Popova, M. B. Oliveira, J. F. Mano, P. A. Levkin, *Adv. Mater.* **2016**, *28*, 7613.
[42] M. Seifermann, P. Reiser, P. Friederich, P. A. Levkin, *Small Methods* **2023**, *7*, 2300553.

[43] J. J. Wiedmann, Y. N. Demirdögen, S. Schmidt, M. A. Kuzina, Y. Wu, F. Wang, B. Nestler, C. Hopf, P. A. Levkin, *Small* **2023**, *19*, 2204512.
[44] M. Tsotsalas, H. Maheshwari, S. Schmitt, S. Heißler, W. Feng, P. A. Levkin, *Adv. Mater. Interfaces* **2016**, *3*, 1500392.
[45] A. N. Efremov, M. Grunze, P. A. Levkin, *Adv. Mater. Interfaces* **2014**, *1*, 1300075.
[46] A. A. Popova, K. Demir, T. G. Hartanto, E. Schmitt, P. A. Levkin, *RSC Adv.* **2016**, *6*, 38263.
[47] A. A. Popova, T. Tronser, K. Demir, P. Haitz, K. Kuodyte, V. Starkuviene, P. Wajda, P. A. Levkin, *Small* **2019**, *15*, 1901299.
[48] H. Cui, X. Wang, J. Wesslowski, T. Tronser, J. Rosenbauer, A. Schug, G. Davidson, A. A. Popova, P. A. Levkin, *Adv. Mater.* **2021**, *33*, 2006434.
[49] J. Bruchmann, I. Pini, T. S. Gill, T. Schwartz, P. A. Levkin, *Adv. Healthcare Mater.* **2017**, *6*, 1601082.
[50] H. Zhang, T. Oellers, W. Feng, T. Abdulazim, E. N. Saw, A. Ludwig, P. A. Levkin, N. Plumeré, *Anal. Chem.* **2017**, *89*, 5832.
[51] W. Lei, J. Bruchmann, J. L. Rüping, P. A. Levkin, T. Schwartz, *Adv. Sci.* **2019**, *6*, 1900519.
[52] A. A. Popova, C. Depew, K. M. Permana, A. Trubitsyn, R. Peravali, J. Á. G. Ordiano, M. Reischl, P. A. Levkin, *SLAS Technol* **2017**, *22*, 163.
[53] M. Jaggy, P. Zhang, A. M. Greiner, T. J. Autenrieth, V. Nedashkivska, A. N. Efremov, C. Blattner, M. Bastmeyer, P. A. Levkin, *Nano Lett.* **2015**, *15*, 7146.
[54] Y. Liu, S. Chakraborty, C. Direksilp, J. M. Scheiger, A. A. Popova, P. A. Levkin, *Mater. Today Bio* **2021**, *12*, 100153.
[55] D. Zahner, J. Abagat, F. Svec, J. M. J. Fréchet, P. A. Levkin, *Adv. Mater.* **2011**, *23*, 3030.
[56] A. B. D. Cassie, S. Baxter, *Trans. Faraday Soc* **1944**, *40*, 546.
[57] Y. Wang, W. Zhao, L. Han, K. C. Tam, *Curr. Opin. Colloid Interface Sci.* **2022**, *57*, 101534.
[58] P. A. Levkin, F. Svec, J. M. J. Fréchet, *Adv. Funct. Mater.* **2009**, *19*, 1993.
[59] T. S. Wong, S. H. Kang, S. K. Y. Tang, E. J. Smythe, B. D. Hatton, A. Grinthal, J. Aizenberg, *Nat.* **2011**, *477*, 443.
[60] L. Wang, T. J. McCarthy, *Angew. Chem., Int. Ed.* **2016**, *55*, 244.
[61] P. J. Bracher, M. Gupta, G. M. Whitesides, *Soft Matter* **2010**, *6*, 4303.
[62] A. Pendurthi, S. Movafaghi, W. Wang, S. Shadman, A. P. Yalin, A. K. Kota, *ACS Appl. Mater. Interfaces* **2017**, *9*, 25656.
[63] Y. Sun, Y. Zhao, X. Xie, H. Li, W. Feng, *Nat. Commun.* **2024**, *15*, 6759.
[64] Y. Zhao, Y. Sun, X. Xie, Y. Liang, E. A. Cavalcanti-Adam, W. Feng, *Adv. Mater.* **2024**, *36*, 2306814.
[65] S. Li, J. M. Scheiger, Z. Wang, Z. Dong, A. Welle, V. Trouillet, P. A. Levkin, *Adv. Funct. Mater.* **2021**, *31*, 2107716.
[66] E. Ueda, F. L. Geyer, V. Nedashkivska, P. A. Levkin, *Lab Chip* **2012**, *12*, 5218.
[67] T. Tronser, K. Demir, M. Reischl, M. Bastmeyer, P. A. Levkin, *Lab Chip* **2018**, *18*, 2257.
[68] W. Lei, K. Demir, J. Overhage, M. Grunze, T. Schwartz, P. A. Levkin, *Adv. Biosyst.* **2020**, *4*, 2000073.
[69] H. Cui, T. Tronser, X. Wang, J. Wesslowski, G. Davidson, A. A. Popova, P. A. Levkin, *Droplet* **2023**, *2*, e39.
[70] H. Cui, X. Sun, M. Schilling, C. Herold-Mende, M. Reischl, P. A. Levkin, A. A. Popova, Ş. Turcan, *Adv. Healthcare Mater.* **2023**, *12*, 2300591.
[71] J. E. Urrutia Gómez, M. Zhou, N. K. Mandsberg, J. A. Serna, J. von Padberg, S. Liu, M. Reischl, P. A. Levkin, A. A. Popova, *Adv. Funct. Mater.* **2024**, 2410355.
[72] Y. Sun, Y. Zhao, X. Xie, H. Li, W. Feng, *Nat. Commun.* **2024**, *15*, 4373.
[73] M. D. Neto, A. Stoppa, M. A. Neto, F. J. Oliveira, M. C. Gomes, A. R. Boccaccini, P. A. Levkin, M. B. Oliveira, J. F. Mano, *Adv. Mater.* **2021**, *33*, 2007695.
[74] J. E. U. Gómez, R. E. K. El Faraj, M. Braun, P. A. Levkin, A. A. Popova, *SLAS Technol* **2024**, *29*, 100118.
[75] M. Zhou, J. E. Urrutia Gomez, N. K. Mandsberg, S. Liu, S. Schmidt, M. Meier, P. A. Levkin, H. Jahnke, A. Popova, *Adv. Healthcare Mater.* **2024**, 2402046.
[76] S. Chakraborty, C. Luchena, J. J. Elton, M. P. Schilling, M. Reischl, M. Roux, P. A. Levkin, A. A. Popova, *Adv. Healthcare Mater.* **2022**, *11*, 2102493.
[77] R. El Khaled EL Faraj, S. Chakraborty, M. Zhou, M. Sobol, D. Thiele, L. M. Shatford-Adams, M. Correa Cassal, A. Kaster, S. Dietrich, P. A. Levkin, A. A. Popova, *Adv. Healthcare Mater.* **2024**, 2401820.
[78] Y. Liu, S. Bertels, M. Reischl, R. Peravali, M. Bastmeyer, A. A. Popova, P. A. Levkin, *Adv. Healthcare Mater.* **2022**, *11*, 2200718.
[79] W. Lei, A. Deckers, C. Luchena, A. Popova, M. Reischl, N. Jung, S. Bräse, T. Schwartz, I. K. Krimmelbein, L. F. Tietze, P. A. Levkin, *Adv. Biol.* **2022**, *6*, 2200166.
[80] A. A. Popova, P. A. Levkin, *Adv. Ther.* **2020**, *3*, 1900100.
[81] A. Letai, P. Bhola, A. L. Welm, *Cancer Cell* **2022**, *40*, 26.
[82] M. J. Iwohn, M. Seifermann, P. Reiser, J. Höpfner, R. El Khaled El Faraj, S. Heißler, A. A. Popova, P. A. Levkin, *Adv. Mater. Interfaces* **2023**, *10*, 2300227.
[83] Y. Tian, M. Seifermann, L. Bauer, C. Luchena, J. J. Wiedmann, S. Schmidt, A. Geisel, S. Afonin, J. Höpfner, M. Brehm, X. Liu, C. Hopf, A. A. Popova, P. A. Levkin, *Small* **2024**, *20*, 2307215.
[84] J. Höpfner, M. Brehm, P. A. Levkin, *Small* **2024**, *20*, 2304325.