TrAC Trends in Analytical Chemistry, Volume 143, October 2021, Article number 116333
DOI:10.1016/j.trac.2021.116333

Droplet microfluidics on analysis of pathogenic microbes for wastewater-based epidemiology

Yangteng Ou[1,2], Shixiang Cao[3], Jing Zhang[1], Weiliang Dong[3], Zhugen Yang[4], Ziyi Yu[*1]

1 State Key Laboratory of Materials-Oriented Chemical Engineering, College of Chemical Engineering, Nanjing Tech University, 30 Puzhu South Road, Nanjing, 211816, P. R. China

2 Yusuf Hamied Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge, CB2 1EW, UK

3 State Key Laboratory of Materials-Oriented Chemical Engineering, Nanjing Tech University, 30 Puzhu South Road, Nanjing, 211816, P. R. China

4 School of Water, Energy and Environment, Cranfield University, Cranfield, MK43 0AL, UK

* Corresponding author. E-mail address: ziyi.yu@njtech.edu.cn

Abstract

Infectious diseases caused by pathogenic microbes have posed a major health issue for the public, such as the ongoing COVID-19 global pandemic. In recent years, wastewater-based epidemiology (WBE) is emerging as an effective and unbiased method for monitoring public health. Despite its increasing importance, the advancement of WBE requires more competent and streamlined analytical platforms. Herein we discuss the interactions between WBE and droplet microfluidics and focus on the analysis of pathogens in droplets, which is hard to be tackled by traditional analytical routines. We highlight research works from three aspects, namely, quantitation of pathogen biomarkers in droplets, single-cell analysis in droplets, and living cell biosensors in droplets, with the aim of providing future perspectives on the synergy between WBE and droplet microfluidics.

Key words: Wastewater-based epidemiology; Droplet microfluidics; Microbiology.

1. Introduction

Tracking population-level public health has been an effective method to identify and assess diseases circulating in the communities, providing early warnings and therefore mitigating their further spreading. Although a number of public health surveillance routes have been established, most of them lack objectivity and are subject to the availability and sophistication of local health services[1]. To this end, wastewater-based epidemiology (WBE) is proposed, an emerging analytical approach that evaluates community-wide public health based on wastewater, to afford comprehensive and unbiased data derived from a given population[2]. WBE is usually carried out upon the extracts from sewage, followed by chemical analysis and data interpretation. The information obtained from wastewater has proved to be useful in the surveillance of infectious diseases, especially in the context of the ongoing COVID-19 global pandemic, where the SARS-CoV-2 RNA was monitored to track community-level infection dynamics[3]. Moreover, WBE was used to study genetic population biomarkers[4], illicit drug consumption[5], propagation of antibiotic resistance[6], holding promises not only in epidemic research but also in providing socioeconomic insights to influence local policy.

In light of its complex compositions, a variety of clinically relevant biomarkers are available for investigation in wastewater, including genetic materials, peptides and proteins, and other chemical or biochemical compounds of interest, which are reviewed elsewhere[1,7]. Microorganism, together with numerous aforementioned molecular markers, is also considered as an important health indicator in the field of WBE[7]. For example, Rengaraj et al.[8] addressed the problem of bacterial contamination in wastewater by employing a paper-based biosensor to quantitate the abundance of microbes thereof, where the interactions between the Concanavalin A-modified carbon electrode and bacterial cells are reflected by the change in electrochemical signals. Furthermore, the profiling of wastewater metagenomics could serve as a powerful tool to monitor infectious diseases[9–11], study the antimicrobial resistance in populations[12,13], track human microbiomes[14], and map the dynamics of bacterial communities[15].

The rapid advancement and the widened availability of analytical methods for WBE have enabled the monitoring of a broad range of biomarkers in wastewater. Paper-based microfluidics, a well-developed analytical tool known for its diagnostic applications[16], therefore attracts attention in WBE mainly due to its capacity for fast and on-site testing, ease of use, and cost-effectiveness. As a result, paper-based biosensors account for the lion's share of the reported analytical devices for WBE, which has been intensively discussed by Mao and colleagues[7,16]. Interestingly, in spite of its increasingly recognized reputation in analytical sciences[17], the droplet-based microfluidics for WBE research have remained largely an untapped area. Nevertheless, droplet microfluidics has in recent decades emerged as a versatile toolkit in many areas, with one of its most pioneering applications being in cell-related research[17]. Consequently, droplet microfluidics should unsurprisingly have great opportunities to be practically utilized to study wastewater, especially in terms of investigating waterborne microorganisms.

In this review, we present the idea of utilizing droplet microfluidics as an analytical platform to investigate WBE, with a specific focus on the analysis of pathogenic microbes. Basics of droplet microfluidics are briefly introduced first to provide a scientific background, followed by discussions on its three different applications, namely, the quantification of biomarkers in droplets, the analysis of microbes in droplets, and living cell-incorporated droplet sensors amid highlighting relevant research works. At last, this review

discusses the practical challenges that might impede the applicability of droplets to wastewater surveillance, aiming to provide insights for WBE researchers to adopt new analytical tools in the future.

2. Basics of Droplet Microfluidics

Microfluidics refers to fluid-handling techniques that manipulate fluids of extremely small volumes, usually ranging from microliters to attoliters[18]. A typical microfluidic experiment is carried out upon a palm-sized chip and uses syringe pump or other pressure-driven systems to displace and control the flow in microchannels, and therefore microfluidics is alternatively recognized as the "lab-on-a-chip" technology. There are two major branches of this science, i.e., continuous-flow microfluidics and droplet microfluidics. The main difference is that the former mostly only involves one phase (continuous phase) whereas droplet microfluidics is at least biphasic with a dispersed phase and a continuous phase in the form of highly monodispersed microdroplets. Regardless of this dissimilarity, scaling laws apply to both systems; as a result, due to the small scale, fluidics are dominated by the viscous force instead of the inertia force, and body forces such as gravity become negligible, thus entering the low Reynolds flow regime[19]. Therefore, fluidics are characterized by laminar flows of which the behaviors are predictable, giving rise to the capacity for precise control and manipulation of fluids.

In the field of droplet microfluidics, droplets can be manipulated either discretely on an array of electrodes or continuously in enclosed microchannels. The former is defined as the "digital microfluidics" which will not be specifically discussed in this review, and one can find more technical details with relevant applications in these reviews[20]. When fluids are operated in the droplet mode in microchannels, droplet formation is fundamentally the result of the interfacial tension between two phases seeking to minimize the interfacial area[19]. Despite many microfluidic device materials[21] and channel geometries[22] that have been explored, the underlying mechanism of droplet generation is intersecting one fluid by another immiscible fluid that acts as the continuous phase (or termed the carrier phase), which leads to the periodic breakup of fluid threads into droplets[22] (Fig. 1). As phase separation is thermodynamically more favorable than dispersion in a liquid-liquid two-phase system, surfactants are usually added as an energy barrier to prevent droplet fusion. From a kinetic point of view, the breaking of capillary instability, also known as the Plateau-Rayleigh instability, directly results in the formation of droplets. When two immiscible fluids meet at the channel junction, the interface deforms under the influence of the viscous and inertia force, while the interfacial tension counteracts to defy this deformation. At some point a force balance is achieved at the interface, and the droplet is formed subsequently. The dynamics of droplet generation in microchannels is reviewed comprehensively elsewhere[19,22]. In droplet microfluidics, key parameters that affect the process of droplet-making include the overall physical properties of fluids (viscosity, density, etc.), flow rates, and channel geometry and surface chemistry, which in turn can be utilized to tailor the droplet size, generation frequency, droplet compositions, etc.

The greatest advantage that microfluidics has over other analytical platforms is its ability to spatially and temporally manipulate the concentration of molecules amid a substantially reduced consumption of samples and reagents[18]. Furthermore, droplet microfluidics affords another dimension of control via compartmentalization, which brings opportunities unparalleled by its bulk counterparts. For instance, the ability to carry out chemical/biochemical reactions in a massive array of droplets simultaneously is highly

prized in the field of analytical chemistry, as it offers better detecting sensitivity and data accuracy owing to the greatly increased number of technical replicates[23]. Another research area that typically exemplifies the inevitability of droplet microfluidics is the emerging single-cell study. Droplet microfluidics has an absolute predominance in this field because it enables the robust encapsulation of cells inside droplets down to single-cell resolution, along with the fine-tuning of droplet compositions for a variety of applications, which is otherwise extremely hard to be achieved. With the advancement in both lab-on-a-chip technology and analytical sciences in the recent decade, it is now possible to execute more complex experimental protocols in microfluidic chips beyond droplet formation, including droplet trapping[24,25], droplet splitting and merging[26], droplet sorting[27], injection of additional reagents into droplets[28], among nearly infinite possibilities.

3. Quantification of pathogen biomarkers in wastewater using droplets

In WBE, detecting pathogen-related biomarkers is vital to identify the diseases circulating within the population[1]. In this regard, probing genetic materials, i.e., pathogenic DNA/RNA residues, has undoubtedly become the focus, as they afford pathogen-wise specificity. Nucleic acid testing in wastewater has been practically used in the surveillance of infectious viral diseases, the examples of which are summarized here[29]. Along with the identification of specific pathogens that cause communicable diseases, nucleic acids in wastewater are able to provide other health information such as carcinogenesis (by quantifying mitochondrial DNA[4,30]) and antimicrobial resistance (by profiling ARGs[12,13]) inflicted on populations.

Another reason why nucleic acid as a biomarker has received considerable attention in WBE is that there are well-established protocols for detecting and quantitating them, and these techniques are readily accessible. Thus far, polymerases chain reaction (PCR) is considered as the golden procedure for the detection of DNA presented in various types of samples, which is ascribed to its capacity for rapidly amplifying the target gene, for example, a pathogen-specific DNA sequence. To investigate the abundance of a specific gene in samples, real-time PCR (rtPCR) was invented. The working principle of rtPCR is to compare the cycle threshold (Ct) of the sample tested with a series of standard samples of known DNA concentrations and hence the DNA concentration in the test sample can be deducted[31]. However, in the case of wastewater, due to its complex contents, many PCR inhibitors presented in the raw sample, e.g., fats, proteins, humic and fulvic acids, etc., might adversely affect PCR results and therefore the accuracy of rtPCR can vary widely[1]. Alternatively, digital PCR (dPCR) has emerged as a better approach for DNA quantification in this situation, which involves molecule partitioning and stochastic mathematical modeling for the absolute measurement of DNA without referring to standard curves[32]. Owing to the partitioning in dPCR, this technique shows higher resilience to those PCR inhibitory substances in environmental samples[33], which makes it more suitable for investigating wastewater in real-life scenarios.

The advent of droplet microfluidic has further revolutionized the dPCR technique and directly gave birth to droplet digital PCR (ddPCR). Essentially, the dPCR method works by first partitioning the reaction mixture into up to thousands of tiny confinements of equal volume, and in ddPCR, this number can go up to tens of thousands or even higher. The DNA molecules in the sample are randomly delivered into each confinement, which is governed by the Poisson distribution below,

$$P(X = k) = \frac{\lambda^k e^{-\lambda}}{k!}$$

where P stands for the theoretical probability of finding k copies of DNA inside one confinement (droplet), and λ represents the average number of DNA copies. These tiny reactors are subsequently subject to thermal cycles similar to the standard PCR procedure until endpoint. Due to the presence of fluorescence probes in the reaction mixture, droplets that contain the target DNA copies fluoresce whereas those devoid of the target DNA do not. Next these samples are checked by a fluorescence detector with a binary readout of positive and negative, and the fraction of positive reactors is recorded. When there is no copy of target DNA in the confinement, that is, k equals 0 in the Poisson mathematical model, the probability of finding at least one DNA copy is given by

$$P(X > 0) = 1 - P(X = 0) = 1 - e^{-\lambda}$$

Usually, a series of dilution is performed on the initial sample prior to partitioning. For different dilution factors d, the probability can be further described as

$$P(X > 0) = 1 - e^{-\frac{\lambda}{d}}$$

At last, by applying linear regression to the fraction (probability P) of positive results as the function of the dilution factor d at logarithmic scale, the absolute number of copies in the original sample can be obtained.

The unique feature droplet microfluidics possesses that distinguishes it from other partitioning approaches, such as chamber-based techniques, is that it can generate miniaturized reactors at ultra-high throughput and of low variances in size[23]. Pinheiro et al.[34] examined a nascently commercialized ddPCR platform (Bio-Rad, 2015) whilst comparing the analytical performance with a cdPCR (chamber digital PCR) instrument that had already existed in the market (Fig. 2A). The authors first demonstrated that the ddPCR platform could achieve a linear dynamic range spanning across 4 orders of magnitude for the absolute measurement of DNA copy numbers. Although the total reaction volume was larger (20 microliters versus 10 microliters), the ddPCR was able to partition the reaction mixture into 20,000 droplets, around 25 times higher than the capacity of cdPCR. The droplet size variance of ddPCR was also found smaller than that of cdPCR, which could reduce the inaccuracy of the measurement[23]. The lower uncertainty in partition volume together with the increase in the number of reactions analyzed improved the overall analytical performance of the ddPCR platform, as the relative expanded uncertainties was fewer than 5% while over 10% for cdPCR.

ddPCR has been successfully applied to detect and quantify the presence of genetic materials from various sorts of sample, especially when external calibrants are inaccessible. One of the maturest applications is the diagnostics of infectious diseases in clinical samples[35,36], and ddPCR demonstrates versality in investigating different pathogenic species including viruses, bacteria, and parasites. Other environmental samples are also reported, for example, quantifying the viable lactic acid bacteria DNA from fecal samples to study the health effects of ingested probiotics[37], and the environmental DNA from aquatic ecosystems to estimate the fish abundance and biomass[38]. Some recent research works also highlight the utility of ddPCR in analyzing wastewater. Jahne et al.[39] for the first time conducted quantitative research on the viral enteric pathogens, namely, norovirus and adenovirus, from on-site collected decentralized wastewaters, providing empirical date to validate their previously reported epidemiology-based simulation[40] and to lend support to further quantitative microbial risk assessment of decentralized water reuse. In the very recent COVID-19 global pandemic, ddPCR has also contributed to the study of population-wide SARS-CoV-2 infection based on WBE in the U.S.[9] and Canada[10], underscoring the ability of WBE to

predict municipal disease outbreak before its occurrence. In addition, ddPCR was also employed to detect SARS-CoV-2 RNA in low abundance wastewater samples such as aircraft wastewater[11] and non-wastewater ones such as clinical samples[41].

As the underpinning principle of ddPCR — the Poisson stochasticity — is not unique, it has enlightened some other field. For instance, Pfammatter et al.[42] developed a droplet microfluidics-based platform, termed as d-AQuA (digital Amyloid Quantitative Assay), for the absolute quantification of aggregation-prone proteins. The self-assembly of proteins into pathogenic amyloid fibrils displays a certain level of similarity to DNA replication, such as the requirement of monomers (soluble proteins), the ability to be detected by fluorescence dye (Thioflavin T, ThT), and the reaction kinetics (exponential growth)[43]. These features underlie the mechanism behind d-AQuA. In this work, samples containing "propagons", defined as all the protein species able to seed and catalyze the conversion of monomeric proteins into higher amyloid structures, are partitioned stochastically into microdroplets along with ThT dyes and the corresponding monomeric proteins. Droplets containing at least one propagon are detected by ThT fluorescence after the propagation. Likewise, the absolute number of propagons presented in the original sample can be estimated. Given that many proteins are found inclined to form amyloid fibrils that share a universal structure irrespective of the initial protein sequence[43], d-AQuA might represent a promising approach in the future to monitor pathogenic protein-associated diseases in environmental samples, for example, prion proteins in wastewater[44].

4. Analysis and monitoring of microorganisms in droplets

It is worth noting that the data derived from most of the analytical methods including ddPCR in the wastewater research reflects only the collective information to which the whole area contributes. However, wastewaters are highly complex and heterogeneous and consist of many different types of microorganisms whose behaviors and genomics are poorly researched. It is therefore necessary to further exploit the wastewater and extract more in-depth information from these waterborne microbes directly and individually. To this end, droplet microfluidics has also offered an excellent solution with regard to the cell-based studies.

Droplets afford a confined space where cells can be encapsulated in a semi-defined manner. In practice, cells are supplied from the reservoir, pushed through the microchannels, and randomly partitioned into the droplets at the channel junction. This process is again governed by the Poisson process[45] and therefore variances in cell number can be observed in droplets. Although the arbitrary controlling of cell distribution is unlikely due to stochasticity, it is feasible to reversely apply the Poisson model for the optimized cell occupancy in droplets[46]. In single cell encapsulation, cell mixtures are usually very diluted to ensure that each cell-containing droplet only has one cell. There are some efforts made to actively bypass the Poisson encapsulation, for example, through hydrodynamic manipulation[47] and through supramolecular crosslinking[48]. These methods are not very suited to analytical applications as they require special design either of the device[47] or of the polymer system and cell pretreatment[48]. One satisfactory solution is to utilize fluorescence activated droplet sorting after cell encapsulation, which is able to enrich cell-containing droplets from the droplet pool. Droplet microfluidics not only greatly advances the cell encapsulation

technique to an unprecedented single-cell level, but also provides an arsenal of experimental tools intended for an extremely wide spectrum of applications.

4.1 Identification of single cells in droplets

Identification of pathogens in wastewater is of paramount importance. As described above, ddPCR is able to detect and quantitate pathogen DNA/RNA residues using the power of statistics, and therefore this method has been expectedly used in a similar way to measure the abundance of a specific microbe both in monocultures and in consortia[49] and of antimicrobial-resistant bacterial cells in blood samples[36] (Fig. 2B). PCR is routinely used in droplet-assisted single-cell analysis, especially for biological profiling[50]; but for only identification purpose, many other non-PCR-based methods have been established. For example, isothermal amplification has emerged as an alternative option other than PCR, because it does not require delicate temperature cycling to amplify DNA/RNA molecules, which represents a major advantage over PCR-based methods. Rakszewska et al.[51] reported DNA-functionalized hydrogel beads to capture mRNA from lysed single cells (Fig. 3A-a). These functional beads contain immobilized primers in the polymeric backbone, which hybridize to the mRNA of interest and are subject to reverse transcription to generate cDNA. Upon hybridization a padlock probe is attached to the cDNA, followed by isothermal rolling circle amplification to yield a long DNA concatemer that can be marked by fluorescent detection oligonucleotides. This work demonstrates that two transcripts from one cell can be detected, and their relative abundance is assessed facilely by counting fluorescent dots. Amplification-free methods have been introduced for single-cell genetic detection in droplets as well. The nucleic acid hybridization lies at the center of these detection routines, where nucleic acid, which serves as the recognition moiety, is flanked by a fluorophore and a quencher at either end. At its natural state, the probe folds and hence little to no fluorescence due to the close vicinity between the fluorophore and quencher. Upon the binding to the target sequence, the quencher is displaced, allowing the probe to fluoresce. Based on this concept, Guo et al.[52] presented a single-cell miRNA detection method in droplets (Fig. 3A-b). In this work, miRNA released after cell lysis catalyzes a hybridization chain reaction between two hairpin DNA (H1 and H2). Subsequently the H1-H2 complex frees the fluorophore from its quencher and hence the increase in fluorescence intensity. The presence of miRNA can be detected within 20 minutes and the customized photomultiplier enables a detection throughput of 300-500 cells per minute. Alternatively, Rane et al.[53] developed a peptide nucleic acid (PNA) beacon to detect 16S rRNA present in single pathogenic cells encapsulated in droplets. More recently, Mach et al.[54] designed an optimized double strand PNA (dsPNA) probe with better sensitivity for identifying bacterial pathogens at single-cell level in droplets (Fig. 3A-c).

To analyze a complex environmental sample of unknown contents, the capacity for accurate and multiplexed detection is highly desired. In order to address this challenge, Xiao et al.[55] developed a droplet-based analytical tool for single bacterial cell detection in a rapid, super-multiplex, and high-throughput fashion (Fig. 3B). This platform works by incorporating stochastic DNA walkers and oligonucleotide-functionalized gold nanoparticles inside droplets alongside single cells. Upon the binding of the target bacterial cell to the aptamer, DNA walkers are released and then trigger a cascade of reactions that leads to the amplified fluorescent signal in the droplets. The super-multiplexed detection capacity arises from two dimensions, i.e., by using different fluorophores (AMCA, FAM, and TR) and by precisely controlling the number

of DNA walkers to adjust the fluorescence intensity (8 intensity levels achieved per fluorophore). Therefore, this method theoretically can barcode 511 (8^3-1) different bacterial combinations and the coding capacity can increase exponentially. The author shows the workability of this platform by analyzing standard samples containing 9 types of bacteria and practical samples, and other advantages including high selectivity to the target cell as well as excellent sensitivity and high throughput which are afforded by droplet microfluidics.

Nucleic acid-based routines are often considered as the first choice for identification as they provide well-defined and highly specific interactions. Aside from DNA/RNA, different types of substances that are able to mark pathogens have been exploited for detecting pathogens in droplet microfluidic settings. For example, Kang et al.[56] managed to identify single *E. coli* cells from unprocessed blood samples in droplets by using a bacteria-specific DNAzyme sensor that emits fluorescence when interacting with *E. coli* lysates (Fig. 3C-a). This work was underpinned by a previous research by Ali and colleagues[57], where they conducted *in vitro* evolution on an RNA-cleaving fluorescent DNAzyme system to obtain this highly selective *E. coli*-targeting DNAzyme probe. According to the results they failed to elucidate the mechanism then but provided substantive evidence that it had involved a protein weighing 30,000 to 50,000 Daltons[57]. Another strategy is to detect secretomics from single cells. Specifically, a work by Lyu et al.[58] demonstrated the detection of *Mycobacterium tuberculosis* (Mtb) in droplets via employing a fluorescent dye that can be digested and activated by secreted BlaC, a highly conserved protein found across all Mtb clinical isolates (Fig. 3C-b). This method showed a high sensitivity and specificity towards the BlaC in droplets over its close class A homologue TEM-1 Bla even in the presence of other bacterial cells of 1 million times more concentrated. Non-fluorescence methods have been developed as well, for instance, by measuring the turbidity arising from single-cell culture in droplets[59]. Zhang et al.[60] innovatively invented a Janus droplet-based approach for the identification of *E. coli*, where the specific interactions between the lectin on cell surface and the mannose immobilized in surfactants are translated into the morphological change in Janus emulsions and the following agglutination. More recently, this method is further advanced by the same research group[61] (Fig. 3C-c). In this work, the boronic acid-modified surfactants form reversible covalent bonds with carbohydrates; when the pathogenic microbes are in presence, dynamics among boronic acids, carbohydrates, and bacterial cells are established, which can be reflected optically because of the altered emission light intensity by varied droplet morphologies.

4.2 Genotypic profiling of single cells in droplets

One of the most game-changing applications of droplet microfluidics is single-cell profiling. Cell biology studies for a long time have been limited by cell population-based techniques and therefore only average properties of cells can be measured. However, cell-to-cell variances are observed constantly in many biological systems[62], let alone an environmental sample that contains different microbial communities. Single-cell studies have been hampered by the lack of techniques capable of effectively isolating individual cells until recent years when droplet microfluidics introduced a revolutionary way of physically separating and confining single cells in tiny micro-reactors, and it soon became the most frequently employed method for single-cell studies[17].

Genomic sequencing by whole-genome amplification (WGA) at single-cell resolution is of particular importance because environmental microbes could exhibit notable genetic heterogeneity even in isogenic populations[62]. Routinely used methods for WGA are always challenged by the inability to obtain a complete genome from single bacterial cells. This problem has been well-addressed by droplet microfluidics. Dichosa and coworkers detailed in this protocol[63] the original work of using gel microdroplets (GMD) to culture single bacterial cells into clonal microcolonies for WGA, from which they managed to obtain near-complete genomes from a microbiome in different environmental samples[64]. Hosokawa et al.[65] adopted a non-culture-based droplet method for single cell WGA, where single cell lysates were passively fused with droplets containing reagents for multiple displacement amplification (MDA). Positive droplets were selectively collected and subject to a second round of MDA to re-amplify the genetic information (Fig. 4A). Both bacterial cells and mammalian cells were compatible with this platform, and the author also showed its applicability in assessing environmental samples. As an alternative, an amplification-free method for single bacterial cell genomic profiling was reported by Lan and colleagues[66] . The workflow includes the generation of three types of droplet pools of different functions, namely, agarose microgels containing fragmented and tagmented genomic DNAs from lysed single cells, droplets containing identical short barcode sequences, and droplets containing PCR reagents. The former two droplets are engulfed by the other one consecutively in the microdroplet maker, and subsequently genomic DNA fragments from a single cell are labeled by the same barcode sequences to allow for pooled sequencing and sorting *in silico*. This pipeline enables genomic profiling of more 50,000 cells per experimental run. In practice, a marine sample from San Francisco coastline was analyzed in this work to profile the antibiotic resistance genes, virulence factors, and phage sequences thereof. Furthermore, Fu[67] et al. took the single-cell WGA in a different direction, in which single cell lysates, instead of the cell itself, were compartmentalized into droplets and genetically amplified by MDA (Fig. 4B). This work displayed a significantly reduced amplification bias as well as improved genomic recovery. Aside from WGA, other single-cell genome-wide profiling works were reported based on droplet microfluidic platform. For example, Thibault et al.[68] coupled single-cell transposon-insertion sequencing (Tn-Seq) with droplet microfluidics, which helped deciphering complicated single-cell phenotypes while obviating the population effects on individual cells. Spencer and coworkers[69] described an epicPCR methodology (emulsion, paired isolation, and concatenation PCR) to profile the target functional gene and associate it with a phylogenetic marker at the same time in a high throughput manner (Fig. 4C).

Transcriptomic profiling of single cells is arguably the most transformative application since the advent of droplet microfluidics. This field gained enormous popularity after the breakthrough made in 2015, where the seminal works of high-throughput inDrop and Drop-Seq were originally developed[70,71] (Fig. 5A and 5B). In these works, single cells from dissociated biological tissues are isolated in droplets individually together with a microbead marked by a droplet-specific barcode with a large number of unique molecular identifier (UMI) for mRNA counts. After the cell is lysed, the released mRNAs are captured by the bead and reverse-transcribed into cDNAs. Then, droplets are demulsified, and the cDNA library is sequenced to generate the transcriptomics of a single cell. As such, the cellular heterogeneity can be mapped by a detailed portray of gene expression status from single-cell resolution. Droplet single-cell RNA sequencing (droplet scRNA-seq) has been successful in characterizing single mammalian cells, such as identifying subpopulations in mammalian organ systems[70–72], constructing temporal dynamics of gene expression

during cell differentiation[73–75], and dissecting cellular responses to environmental stress[76,77], with many other more exciting sciences yet to be discovered.

Despite the huge success of scRNA-seq for profiling transcriptomics in eukaryotes, these methods, including droplet scRNA-seq, has not been widely adapted for microbes[78]. To be specific, bacterial mRNA is not polyadenylated, which affects the capture efficiency by beads and separation from ribosomal RNA. Moreover, microbes have much lower mRNA availability than human cells and the cell wall poses challenge for lysis[78]. A straightforward protocol for bacterial mRNA polyadenylation is to employ *E. coli* poly(A) polymerase I (PAP) that adds the 3' polyadenine tail to mRNA, which was demonstrated in a recent work by Kuchina et al.[79], where the mRNA was markedly enriched after reverse-transcribed with primers containing barcoded poly-T to generate cDNA library (Fig. 5C). To circumvent the need for polyadenylation in bacterial mRNA sequencing, Imdahl and coworkers[80] applied the multiple annealing and dC-tailing-based quantitative scRNA-seq methodology (MATQ-seq), a derivative of MALBAC (multiple annealing and looping-based amplification cycles) method that covers the whole RNA region[81] (Fig. 5D), to profile the transcriptomics of *Salmonella* under different growth conditions. Although this work used FACS for single cell isolation, the author did envisage a droplet microfluidics-enabled platform for the throughput scaling-up and cost reduction in the future. Another problem associated with microbial scRNA-seq is the low contents of mRNA that is reported to be around two orders of less than the human cells[78]. The challenge was tackled by culturing single cells inside cytocompatible microgels[82] before lysis and scRNA-seq, which resembles the aforementioned GMD method for WGA[63]. Using the standardized Drop-Seq approach[70], they managed to map the heterogeneity in gene expressions from a protein mutagenesis library in over 1,000 engineered *Saccharomyces cerevisiae* single cells (Fig. 5E).

4.3 Phenotypic testing of single cells in droplets

Single-cell genotypic profiling indeed provides a wealth of deep genetic information; however, bioinformatics cannot always predict functions[83] and transcription status is not the sole factor dictating protein translation because of complex epigenetic regulations. Therefore, direct testing of phenotype is necessary for investigating protein functions and for probing genotype-phenotype distinction.

Droplet confinements allows for assessing cell behaviors in real time and in high throughput. For example, Shim et al.[24] reported a microfluidic strategy to immobilize single cell-laden droplets that enables them to monitor the enzymatic activity of alkaline phosphatase (AP) secreted by single *E. coli* cell in droplets (Fig. 6A). In addition, after normalizing the enzymatic performance by the co-expressed mRFP1, they discovered notable fluctuations in AP levels expressed from the same plasmid. Kehe and coworkers[25] introduced a sophisticated droplet platform, kChip, to precisely group and manipulate *k* droplets in microwells for monitoring and screening bacterial phenotypes. Through tailoring the droplet contents, they were able to characterize bacterial phenotypes across various environmental conditions, and more significantly, to perform combinatory screening of multispecies communities (Fig. 6B). Furthermore, droplet microfluidics also enables large-scale testing of cellular responses to chemicals, from which emerges one of the most important applications of droplet microfluidics, namely, drug screening and drug discovery[84–86] (Fig. 6C).

It is important to point out that droplet single-cell study has an intrinsic pitfall — the Poisson statistics. Under the most ideal condition, the highest single-cell occupancy (where λ equals 1) is around 37% of all the droplets generated, leaving a large portion of droplets unwanted. Therefore, there has been an urgent need for an effective sorting method to recover the desired droplets for downstream processing. Since it has a technological importance for many other fields, single cell sorting has witnessed an array of enabling tools involving different mechanisms, such as electrokinetics, acoustophoresis, optics, mechanics, hydrodynamics, and magnetophoretics, which are summarized and intensively discussed this review[87].

As one of the major derivative techniques of droplet microfluidics, Fluorescence Activated Droplet Sorting (FADS) has emerged as a useful tool for single-cell sorting. Compared with the FACS that the cell fluorescence can only be detected either inside the cell or at the surface, the confinement of droplets brings the unique opportunity to examine not only the cell itself but also molecules secreted by the cell. Baret et al.[88] in 2009 for the first time reported the droplet system to sort bacterial cells on the basis of enzymatic activity at single cell-level (Fig. 7A). The system is first benchmarked by sorting droplets containing varying concentrations of fluorescein and then utilized to sort *E. coli* cells expressing active/inactive β-galactosidase. It should be noted that β-galactosidase is an intracellular enzyme catalyzing the formation of fluorescein from fluorogenic substrates in the droplets, so the fluorescence signal is raised from a minority of non-viable cells in small clonal populations inside droplets. In order not to affect cell viability and/or other functions, targeting cellular secretomics is a favored strategy. In this protocol[46] a type of antibody-secreting cell is exemplified. After incubation, antibodies produced by the single cell are captured by the co-encapsulated microbead and then labeled by a fluorescent secondary antibody to facilitate sorting. Ostafe et al.[89] reported an approach to sort single yeast cells actively secreting cellulases, where the substrate, cellulose, is decomposed into monosaccharides that triggers a cascade of enzymatic reactions leading to fluorescence (Fig. 7B). Furthermore, Wang and colleagues[90] described a flexible sorting strategy by using two enzymes in tandem to convert the target metabolite into fluorescence signal. In this way they demonstrated its feasibility to select both high producer and high consumer microbes using different combinations of substrate and enzymes. Notably, most of the reported works for sorting secretory phenotypes are built on a similar idea where the target metabolites are enzymatically converted into detectable fluorescence signal. However, not every target molecule can be easily coupled to a fluorescence assay, which undoubtedly hampers the generality of FADS. To tackle this issue Abatemarco et al.[91] presented a RAPID (RNA aptamer in droplets) method capable of responding to a range of target analytes including small molecules and proteins by adjusting aptamer sequence, providing a feasible routine to further expand the applicability of FADS for phenotype-based sorting (Fig. 7C). Besides secretomics, sorting can also be performed by label-free methods via intrinsic fluorescence, such as microalgae and cyanobacteria[92] and plant cells[93]. Other detection schemes, such as Raman-Activated Droplet Sorting (RADS)[94] and Absorbance-Activated Droplet Sorting (AADS)[95], have been proposed to provide more options.

The ability of FADS to carry out high-throughput single-cell sorting based on cell phenotypes has transcended the traditional screening techniques, with one of the most benefitted area being directed evolution (Fig. 7D). Screening a large mutant library is extremely labor-intensive, time-consuming, and costly via conventional plate-based protocols, with successful hits depending on the odds due to the limited throughput. This situation has been completely transformed by FADS. The FADS-enabled directed evolution normally involves the iterative phenotype screening, largely based on a specific enzymatic activity, of single

microbial cells (typically *E. coli* or yeasts) transfected by a mutant library. Owing to the greatly enhanced screening throughput and single-cell manipulation, the likelihood of isolating mutants with desired phenotypes has remarkably increased. Around one year after the first publication of FADS by the Weitz group[88], they applied this platform to screen a mutant library of horseradish peroxidase[96] from which they identified novel mutants exhibiting an over 10-fold increase in enzymatic activity than their parent, with a cost decreased by a million-fold compared to the most advanced robotic screening system at that time. FADS has ever since been employed as a new tool for ultra-throughput engineering of various enzymes including hydrolases, aldolases, esterase, polymerases, oxidoreductase, etc., which are detailed in these reviews with their respective reaction mechanisms[97,98]. FADS has also facilitated the study of functional metagenomics (Fig. 7E). For example, Hosokawa et al.[99] used GMDs coupled with FADS to screen a metagenomic library constructed from soil samples containing 67,000 clones, leading to the uncovering a new lipolytic enzyme. Najah and coworkers[100] screened an environmental sample from a wheat bubble field using FADS with a fluorogenic cellobiose as the bait substrates, in which they isolated a bacterial population with 17- and 7-fold higher cellobiohydrolase and endoglucanase activity, respectively. A more prominent work by Colin et al.[83] showed that they managed to identify 14 new and rare hydrolases with promiscuous activities from a metagenomic library containing over 1 million clones constructed from soils and vanilla pods. These works perfectly epitomize the power of FADS as a new instrumental format for researching environmental genomics, which would otherwise be extremely challenging to address without the throughput gains afforded by microfluidics.

Another application of droplet microfluidics for single cell phenotype testing that might be of interest to wastewater researchers is investigating microbial antibiotic susceptibility, as wastewater is recognized as a breeding ground for antibiotic-resistant microbes[15]. Antimicrobial resistance has increasingly been a global concern, posing a major public health threat worldwide. Although profiling antimicrobial resistance genes, for example, mecA gene, through PCR-based methods could enable the identification of such species, not every antibiotic-resistant strain can be genetically marked[101]. Droplet single-cell microbial antibiotic susceptibility assay was introduced by Boedicker [101] et al. in 2008, where they portrayed the antibiotic sensitivity of a methicillin-resistant strain, *Staphylococcus aureus*, to 6 antibiotics as well as to different concentrations of cefoxitin to determine the minimal inhibitory concentration (MIC). The applicability of this method to environmental samples was validated by detecting bacteria in human blood plasma. Kaushik et al.[102] designed an integrated microfluidic chip where single bacterial cell encapsulation, incubation, and detection could all be performed in one chip, accelerating the assessment of bacterial growth and antibiotic susceptibility. As a demonstration, they tested the antimicrobial effect of gentamicin against *E. coli* within one hour with resazurin as the viability marker. By incorporating fluorescent bacterial viability indicators, this platform is well compatible with FADS. For instance, Eun and colleagues[103] measured the MIC of rifampicin against singularly encapsulated *E. coli* cells in agarose microgels; using the expression of EGFP as a live/dead indicator in FADS, they isolated mutants that had developed resistance to rifampicin, and further DNA sequencing of such mutant bridged the space between this phenotype and its genotype. For environmental applications, Terekhov et al.[104] profiled the antimicrobial activity of microbiota communities extracted from Siberian bears using droplet single-cell sorting, where they recovered a naturally sourced microbial killer *Bacillus pumilus* strain against *Staphylococcus aureus*. They later performed detailed omics analysis on the *Bacillus* strain and elucidated the mechanism behind its antimicrobial effects.

Liu et al.[105] proposed a label-free FADS mechanism for identifying antibiotic-resistant bacteria, which is established on that because such strains have better resistance to antibiotics, they can proliferate in the droplets and hence more pronounced light scatter thereof. To prove this concept, they tested and isolated fusidic acid-resistant *E. coli* mutant populations, which the authors found to possess one or two mutated sites in their fusA gene in the later DNA sequencing. This approach promises to be a general routine for isolating mutant strains with higher resistance to environmental stress. Other physical principles exclusive of optics, although not routinely applied, also hold promises in investigating microbial sensitivity to antibiotics. For example, magnetism underlies a research work by Sinn et al.[106] in which an asynchronous magnetic bead rotation (AMBR) biosensor measures the bacterial growth under the influence of antibiotics. The growth kinetics of *E. coli* attached to the magnetic bead is translated into its rotational response, so the antimicrobial effects can be physically represented.

5. Living cell biosensors incorporated with droplets

Certainly, there are numerous sensor technologies created for analyzing wastewater[7], or in a larger context, for environmental monitoring[107]. To capture the temporal and spatial dynamics of the contamination in the environment, miniaturized and portable devices are favored. In this respect, the concept of whole-cell biosensor is introduced for environmental applications[108]. The main rationale, besides apparently smaller sizes, for deploying living cells/microorganisms in sensors is that they are able to elicit biological effects supposed to occur in living organisms exposed to the environment[109]. When properly designed, these biological effects can be reflected by readable signals[108] (Fig. 8A). These microorganisms are therefore frequently referred to as “bioreporters”.

Most of the bioreporters leverage specific gene circuits to convert target molecules into the expression of the reporter gene which is either directly fused to or associated with a promoter controlled by the target[108]. The outcome of such interaction is proportional to the abundance of the target molecule in the assayed sample, allowing for the establishment of standard curves from which the quantitative information can be extrapolated[109]. Compared with other analytical devices, microorganisms have several advantages: first, they can self-reproduce themselves rapidly and consequently the analytical ability can be passed on to the next generations; second, microbes provide a natural confinement isolating the reporter gene from the environment and therefore this functional part is more resilient to external disruptions; third, it is possible to detect other organisms via intercellular interactions that might not be well-characterized, potentially ushering in a new direction for detecting pathogenic microbes in environmental implementations. Many immobilization strategies have emerged to host the whole-cell biosensor whilst sustaining its viability and functionality. While Roggo et al.[109] reviewed some of the miniaturized and integrated devices in this regard, in this paper, we will highlight the microorganism-incorporated droplet biosensors.

The major incentive for utilizing droplets to accommodate the living-cell biosensor lies in the flexibility and controllability of droplet microfluidics that facilitate manipulation as well as enhanced sensitivity resulted from miniaturization. For example, Buffi and coworkers[110] trapped agarose beads housing an arsenic bacterial bioreporter in a microfluidic cartridge, making it easy to handle and keep the analytical equipment for actual field applications. Gel droplet biosensors could be arrayed in a microchip, as demonstrated by Ahn et al.[111], where they were able to perform toxicity detection and screening on genetically

engineered *E. coli* capable of sensing five environmental stresses (Fig. 8B). Moreover, droplet microfluidics enables facile co-encapsulation of different cells. Meyer and colleagues[112] co-confined the producer cells of *B. subtilis*, and the sensor cells of *E. coli*, into microdroplets. With microfluidics, the producer cells were isolated at single-cell level whilst surrounded by excessive sensor cells so that the authors managed to identify cells with high production efficiency from a large mutant library. Droplet living-cell biosensor can also be integrated with other functional microfluidic units such as FADS. For instance, Mahler et al.[113] employed microbial biosensors to detect the antibiotics synthesized by a variety of actinobacteria. The antimicrobial potential was indicated by the expression level of fluorescent protein GFP by reporter cells picoinjected into the droplets. Based on this they were capable of identifying high antibiotic-producing actinobacteria using FADS. At last, to improve the stability of droplets and the living-cell biosensors within, hydrogel enhancement approaches were reported. Li et al.[114] encapsulated bacterial reporters in alginate-methacrylate (alginate-MA) microgels for detecting quorum sensing-related molecules; after the first ionic crosslinking of the alginate-MA microgels upon the formation of cell-laden droplets, a secondary photopolymerizing was initiated to increase the crosslinking density and hence the reduced leakage of cells from microgels (Fig. 8C). Inspired by the naturally occurring biosilicification process, Zhao and coworkers[115] reported a strategy to enhance the microgel mechanical property by surface-coating a mesoscopic silica shell onto the microgel surface (Fig. 8D); in this way, the cell leaking problem was profoundly prevented. Furthermore, the silica shell also could serve as a machinery to control the permeability of molecules to the microgel. *E coli* biosensors were embedded in the mechanically enhanced microgels and responded to target molecules at physiologically relevant concentrations as well as to adjacent pathogens.

6. Challenges

6.1 Challenges of analyzing wastewater

WBE has appeared as a relatively new tool for monitoring public health, with its important roles already validated in providing population-wide information on consumption of drugs[2] and more recently in surveilling infectious diseases[1]. As an increasing amount of attention has been drawn to WBE, it is anticipated that more valuable information would be extracted from wastewater and used as the basis for disease prevention and intervention in the future. However, analyzing wastewater poses challenges for WBE.

First and foremost, the complexity of wastewater is a double-edged sword. On the one hand, it provides an extremely diverse availability of biomarkers to profile the health information derived from a population[116]; on the other hand, the presence of other non-target molecules could interfere with the detection. For example, Morales-Belpaire et al.[44] studied the amyloid fibrils formed by lysozymes in wastewater and used ThT to qualitatively indicate the propagation. It transpired that in the presence of wastewater sludge flocs the ThT fluorescence decreased as the function of time, which they ascribed to the hydrolysis of fibrils by proteases in the sludge. Moreover, in a recently reported investigation of SARS-CoV-2 RNA from wastewater solids[10], the authors discovered that the inhibitory effects were found to be more notable on ddPCR than qPCR, in spite of that many other research works had claimed the opposite[37,39,41]. The actual cause was not fully studied in this work, which the authors presumed to be 1) the increased sensitivity of ddPCR to inhibition in wastewater samples due to partitioning when the concentration

approached to the limit of detection, and 2) differences in the reagents used for two assays. Although this might be an individual case, this study raises concerns with regard to using even well-established experimental protocols to analyze wastewater samples and underscores the necessity of further optimizing these methods for environmental monitoring.

Another challenge of analyzing wastewater is uncertainties associated with the dynamics of population, the local environment, and operation conditions[1], which confound the results. The standard approach for estimating population size is to measure the abundance of human endogenous markers in wastewater samples, known as the population markers, and they are then used as to normalize the population size[117]. However, there are problems estimating population size based on wastewater treatment plants catchments (WWTP), especially in small areas that are easily influenced by population fluctuations[1]. The local environment around WWTP also contributes to data uncertainty, as other living non-human organisms might contaminate the sewage system. At last, uncertainties may also arise from prior to the analysis, such as the sampling, extraction, storage, shipment, and other operations[7].

6.2 Limitations of droplet microfluidics

The rapid advancement of WBE requires sophisticated analytical platforms. Paper-based sensor devices provide on-site and nearly real-time detection of target molecules in wastewater, however, at the sacrifice of in-depth data and the capacity for handling microorganisms. Though not widely employed in wastewater research thus far (except for ddPCR), droplet microfluidics can represent a new direction in this field given that there have been numerous successful examples of analyzing other environmental samples, including soil[65,83,99,100,113], aquatic[9–11,38,39,77], animal [37,104], and clinical samples[35,36,41,55,56,58], across a wide range of applications.

There is little doubt that droplet microfluidic platform has afforded elegant solutions to a range of challenges in different fields; nevertheless, it still has some practical and technical limitations[17]. First, droplet platform tends to give multiplexed and detailed analysis rather than simple detection and measurement of target molecules; as a result, like many other traditional analytical techniques used in WBE including mass spectroscopy, PCR, etc., droplet microfluidics cannot offer real-time results. This also indicates that experiments are unlikely to be performed on-site. Central laboratory facilities are required to conduct droplet microfluidic experiments and even more advanced equipment for complicated protocols such as optical measurements, ddPCR, FADS, and DNA sequencing. Second, not every assay can be performed in droplets. The nature of pre-defined confinements hardly allows for a total exchange of droplet contents with the environment, limiting its applications, e.g., where multi-step testing is required. Third, droplets are not perfectly stable even stabilized by surfactants when exposed to different situations. The complex composition of wastewater and external physical conditions such as high temperature in the PCR thermal cycles might disrupt droplet integrity. Fourth, there is still a lack of standardized protocol of droplet microfluidic platforms for cost-effective, selective, sensitive, and multiplexed analysis of microorganisms in wastewater. Newly developed techniques can partially address this challenge from different angles, but they do not hold universality yet. Therefore, multidisciplinary efforts should be made to establish the guidelines for WBE in the future to avoid bias from the technological perspective.

Alongside the technical limitations of the droplet platform, another challenge lies in the pretreatment of wastewater samples for microfluidic analysis. On the one hand, the bulk wastewater samples usually need preconcentrating before handled by droplet microfluidics because of low concentrations of target analytes in collected wastewater with volume of tens of liters; on the other hand, effective and efficient analysis of microbes from wastewater necessitates a purification process prior to cell encapsulation in droplets. This challenge has been addressed microfluidically by integration of automated pretreatment modules onto the device which eliminates the labor-intensive operations and prevents the sample contamination or loss. Examples include the integration of capillary electrophoresis, micellar electrokinetic chromatography, solid phase extraction, etc.[118–120]. Those methods allow the microfluidic device for continuous concentration and capturing of microbes from collected wastewater; however, there is still a need for improving the capture rate and efficiency.

7. Conclusion and Perspectives

The last few decades have witnessed a rapid development of droplet microfluidics with an incredible speed of expansion in different areas. Now droplet microfluidics has mostly become an application-oriented technique, steered by the end-users to a sea of unanswered science questions. Herein in this review, we proposed a new direction – wastewater-based epidemiology, which is yet to be addressed by droplet microfluidics.

Droplet microfluidics engenders two brand new opportunities for WBE. First, the detection of pathogen biomarkers in a more accurate and sensitive manner by ddPCR, and second, the unprecedented high throughput single-cell analysis. Due to the commercialization of ddPCR, it has been recognized as a basic experimental tool and used in monitoring pathogens in wastewater. Single-cell analysis in the context of wastewater has remained conceptual, and there are currently not sufficient works for us to review here; however, in a broader research topic, in light of the fundamental changes that droplet microfluidics has brought to the single-cell studies, there are a number of research works involving the investigation of other environmental samples based on droplet single-cell platform, which we think can be mirrored in wastewater research in the foreseeable future. At last, we reviewed some strategies of utilizing the droplet as the vehicle to host microbial whole-cell biosensors, as we believe this might also provide insights for designing new sensor devices for wastewater surveillance. In conclusion, we are strongly convinced that droplet microfluidics can bridge the unfilled technological gap that are unable to be addressed by other techniques in WBE, holding promises to truly unfold the power of modern wastewater research.

Author contributions

Yangteng Ou: Conceptualization, Writing-original draft, Writing review & editing. **Shixiang Cao**: Writing review & editing. **Jing Zhang**: Writing review & editing. **Weiliang Dong:** Writing review & editing., **Zhugen Yang**: Writing review & editing. **Ziyi Yu:** Conceptualization, Writing - original draft, Writing - review& editing, Project administration.

Declaration of Competing Interest

The authors declared no conflict of interest.

Acknowledgement

The authors would like to acknowledge the support of the National Key R&D Program of China (2019YFA0905500), the National Natural Science Foundation of China (21901117), the Natural Science Foundation of Jiangsu Province (BK20190674), State Key Laboratory of Materials-Oriented Chemical Engineering (ZK201809), Jiangsu Specially-Appointed Professor Program, Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD). Zhugen Yang acknowledge support from a UK NERC Fellowship (NE/R013349/2), a NERC grant (NE/V010441/1) and a Royal Academy of Engineering Frontier Follow-up grant (FF\1920\1\36)

Figures and captions

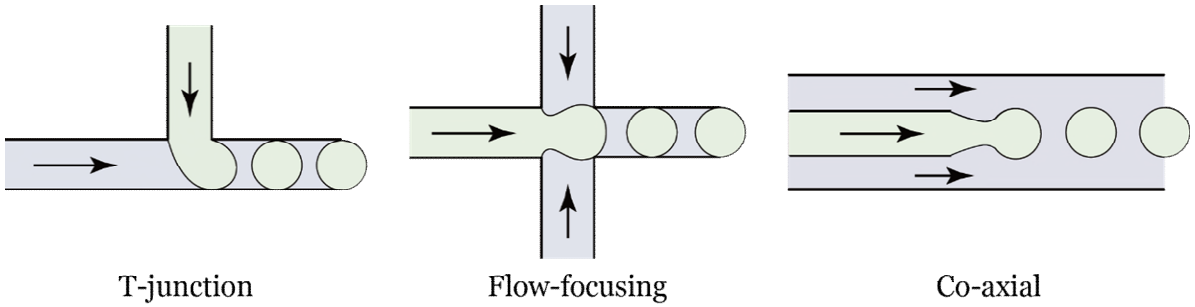

Fig. 1. Common microchannel geometries for droplet generation. Three types of mostly used channel shapes, namely, T-junction, flow-focusing, and co-axial. In all three configurations, droplets are passively generated as the result of fluid instability.

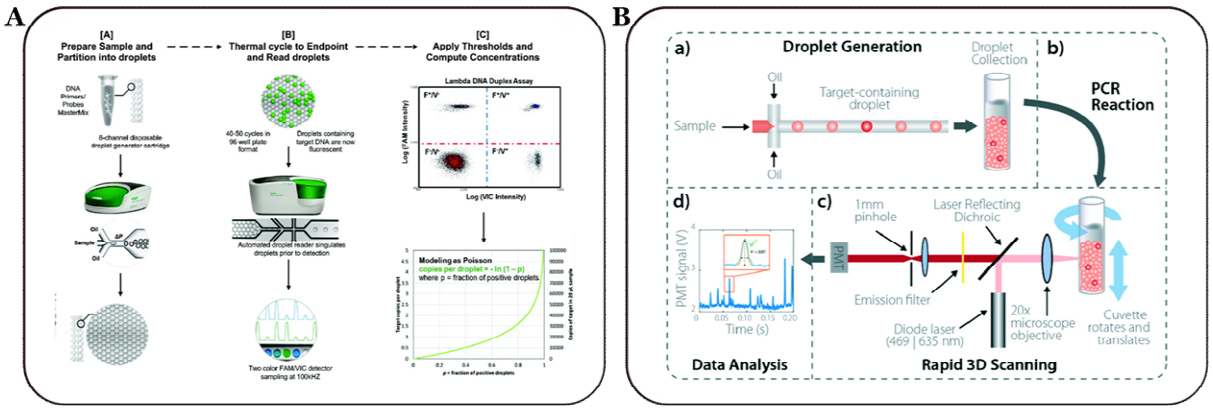

Fig. 2. Droplet digital PCR (ddPCR) for quantification of DNA molecules and detection of pathogenic microbes. (A) Schematic diagram of the ddPCR workflow. (B) Schematic illustration of the Integrated Comprehensive Droplet Digital Detection (IC3D) platform for the detection of pathogenic microorganisms in human blood plasma: (a) single bacterial cells are encapsulated into the droplets and lysed before (b) PCR is performed to detect pathogen-specific genes. (c) A customized scanning platform for high-throughput data reading and (d) data processing. Fig. 2A and 2B are reproduced from the reference [34] and [36], with permissions from (A) American Chemical Society 2011 and (B) The Royal Society of Chemistry 2020.

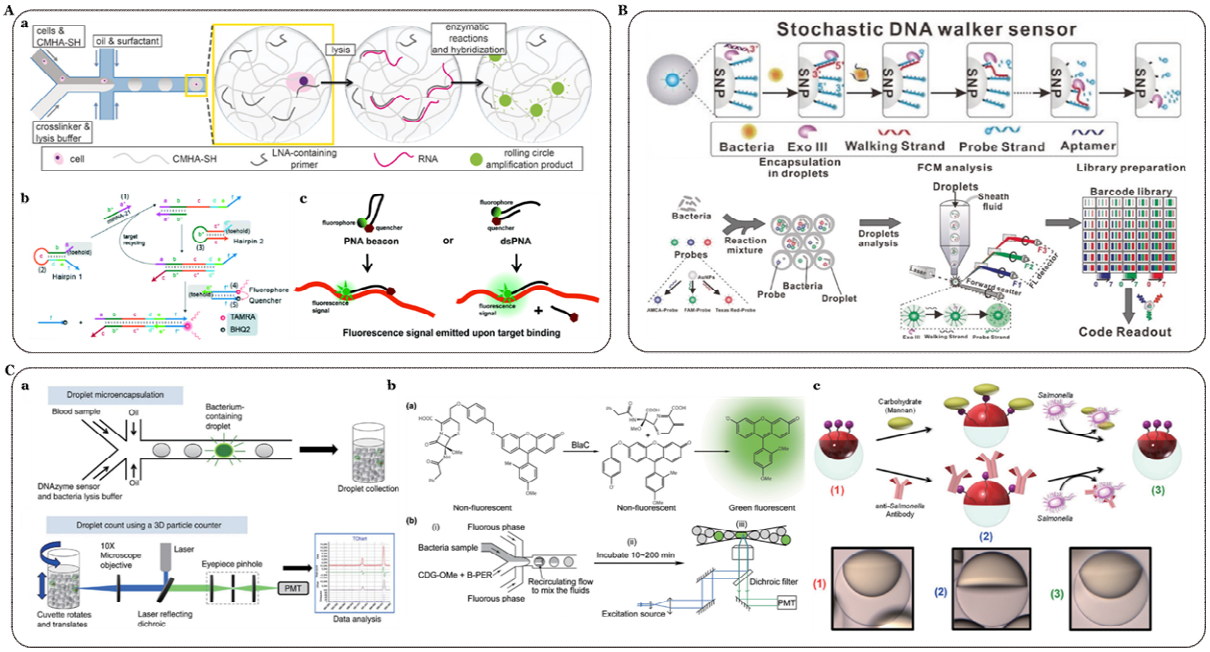

Fig. 3. Identification of pathogenic microbes in droplets. (A) Non-PCR methods for genetic identification of microorganisms. (a) Illustration of an isothermal rolling circle amplification method for visualizing single-cell mRNA in gel microdroplets. (b) Detecting miRNA in droplets. The target miRNA can catalyze the formation of hairpin1-hairpin2 complexes which further separate the TAMRA fluorophore from the quencher and hence the amplified signal in droplets. (c) Mechanism of peptide nucleic acid (PNA) probes. Red ribbon-shaped thread represents the target nucleic acid. Figures are reprinted from reference [51], [52], and [54], with permissions from (a) WILEY 2016 and (b) and (c) the Royal Society of Chemistry 2018 and 2019, respectively. (B) A stochastic DNA walker sensor for multiplexed and high-throughput detection of bacterial pathogens in droplets. Fig. 3B is adopted from reference [55] with the permission from WILEY 2019. (C) Biomarkers other than genetic materials for microbial identification. (a) Schematic showing the applicability of the DNAzyme probe to detect pathogens in blood sample. (b) Identification of pathogens by secretomics. (c) A Janus droplet strategy for detecting foodborne pathogens *Salmonella enterica*. Fig. 3C. are reprinted from reference [56], [58], and [61], with permissions from (b) American Institute of Physics 2015, (c) American Chemical Society 2019, and (a) under a Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/).

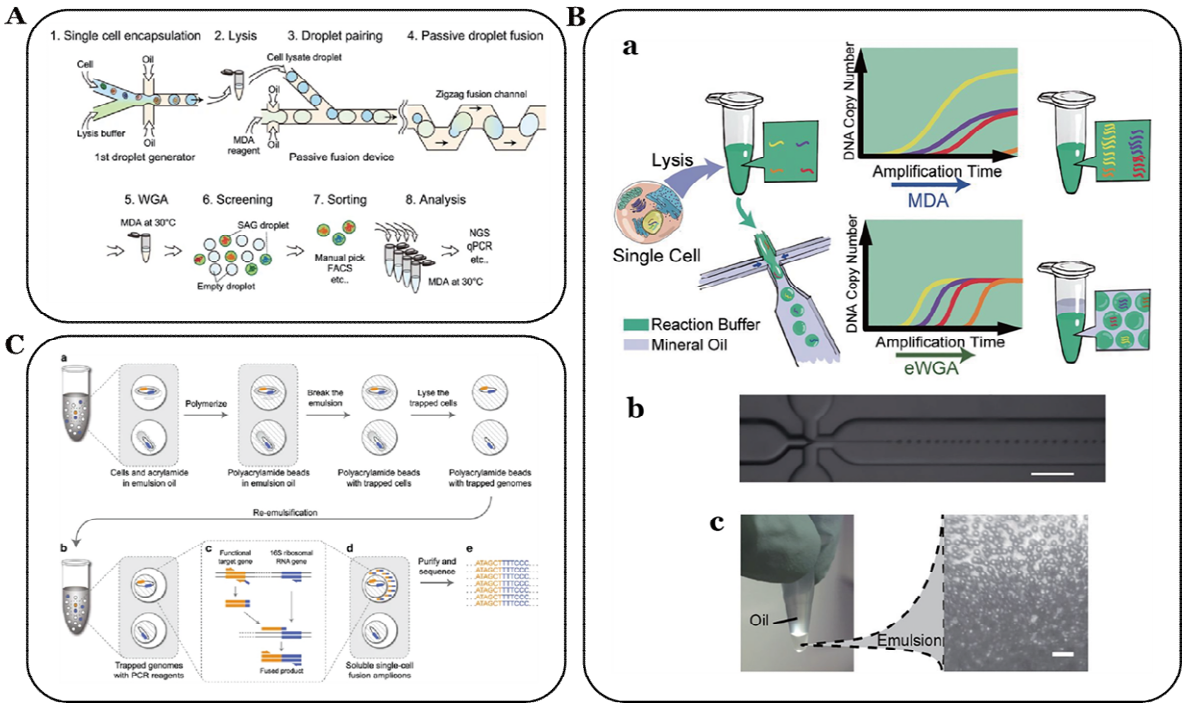

Fig. 4. Genomic profiling of single cells for microbiology in droplets. (A) Two-step WGA of single bacterial cells in droplets. (B) Emulsion WGA sequencing (eWGA-seq) technique. (a) Single cell lysates are partitioned into droplets. (b-c) Pictures of the droplet-maker (b, scale bar 300 μm) and generated emulsion (c, scale bar 100 μm). (C) Workflow of epicPCR (emulsion, paired isolation, and concatenation PCR). (a) Single cells are embedded in polyacrylamide microbeads and then lysed to expose the genomic DNAs. (b) Microbeads are re-emulsified into PCR-containing droplets for fusion PCR, where (c) 16S ribosomal RNA is fused with the functional gene to form the concatenated product. (d) Only when the given microbial cell possesses the target gene can the fused amplicons form. (e) Sequencing reveals the link between the phylogenic marker and the functional gene. Fig. 4A to 4C are adopted from reference [65], [67], and [69], with permissions from respectively, (B) National Academy of Sciences 2015, (A) and (C) under the a Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/).

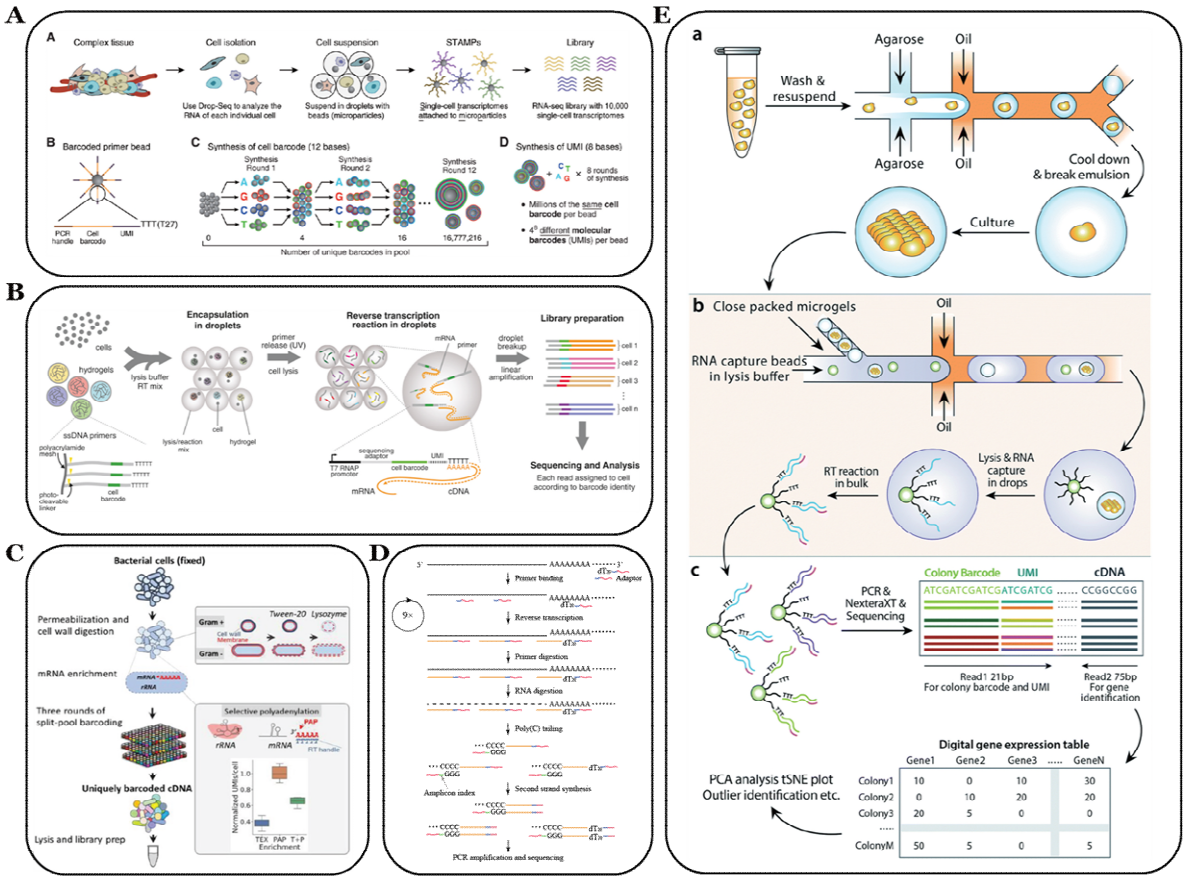

Fig. 5. Transcriptomic profiling of single cells. (A-B) Drop-Seq (A) and inDrop (B) technique for single-cell transcriptomic analysis. Cells are dissociated and encapsulated either (A) with a barcode microbead or (B) in a barcode microgel. (C-D) Strategies for barcoding single prokaryotic cells. (C) MicroSPLiT workflow. Bacterial cells are fixed and permeabilized and mRNA is reverse-transcribed into cDNA inside cells. Then cells are pooled-and-split several rounds for barcoding single cells. (D) The Multiple Annealing and dC-Tailing-based Quantitative single-cell RNA-seq (MATQ-seq) for single-cell transcriptomic profiling. (E) Schematic illustration of utilizing Drop-Seq to profile transcriptomics of yeast colonies from single-cell culture in agarose microgels. Fig 5A to 5C, and 5E are reproduced from reference [70], [71], [79], and [82] with permissions from (A) and (B) Cell Press 2015, (C) American Association for the Advancement of Science 2021, and (D) the Royal Society of Chemistry 2019.

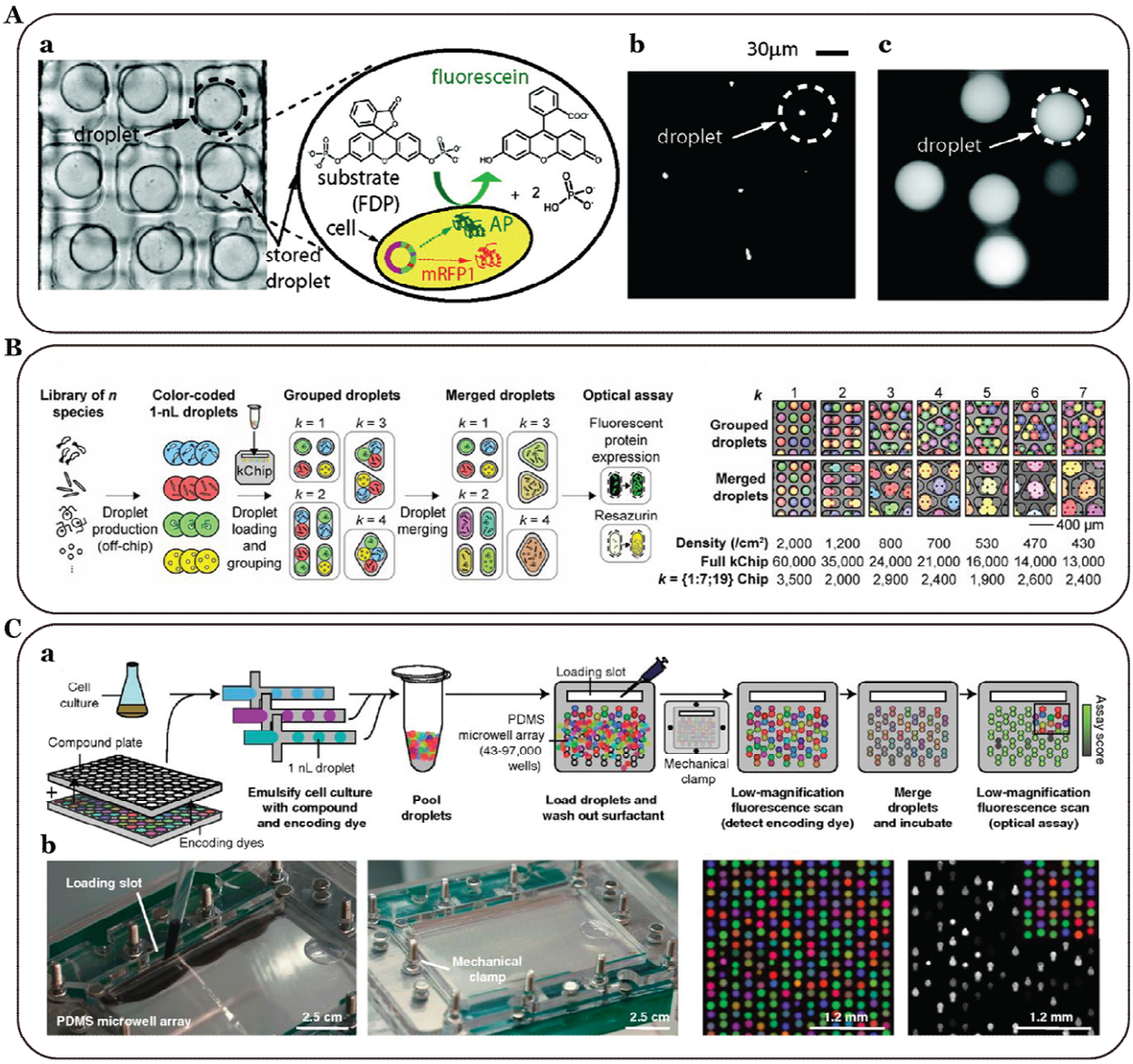

Fig. 6. Phenotypic testing in droplets. (A) An integrated microfluidic chip allowing for real-time and *in situ* monitoring of enzymatic activity. (a) Microscopic picture of entrapped droplets containing single cells. (b) Fluorescence image of mRFP1 expression in droplets, which is used to benchmark the AP expression. (c) Fluorescence image showing the accumulation of fluorescein, indicating the enzymatic activity of AP. (B) Schematic of the workflow of a droplet platform, kChip, for large-scale phenotypic characterizing and screening of synthetic microbial communities. Microorganisms are encapsulated in droplets and color-coded before loaded into the kChip containing tens of thousands of microwells that enable the grouping of *k* droplets. Droplets in the same microwell are merged upon the short exposure to an alternating-current electric field. Optical measurements are then carried out to characterize phenotypes. By pooling and then randomly grouping droplets containing different microorganisms, multispecies communities phenotypic testing and screening can be performed in parallel. (C) Combinatory drug screening using droplets. (a) Schematic illustration of the workflow. (b) Microscopic and fluorescence images of the microfluidic chip and GFP expression from arrayed bacterial cells in merged droplets. Fig. 6A to 6C are reprinted from reference [24], [25], and [86], with permissions from (A) American Chemical Society 2009 and (B) and (C) National Academy of Sciences 2019 and 2018, respectively.

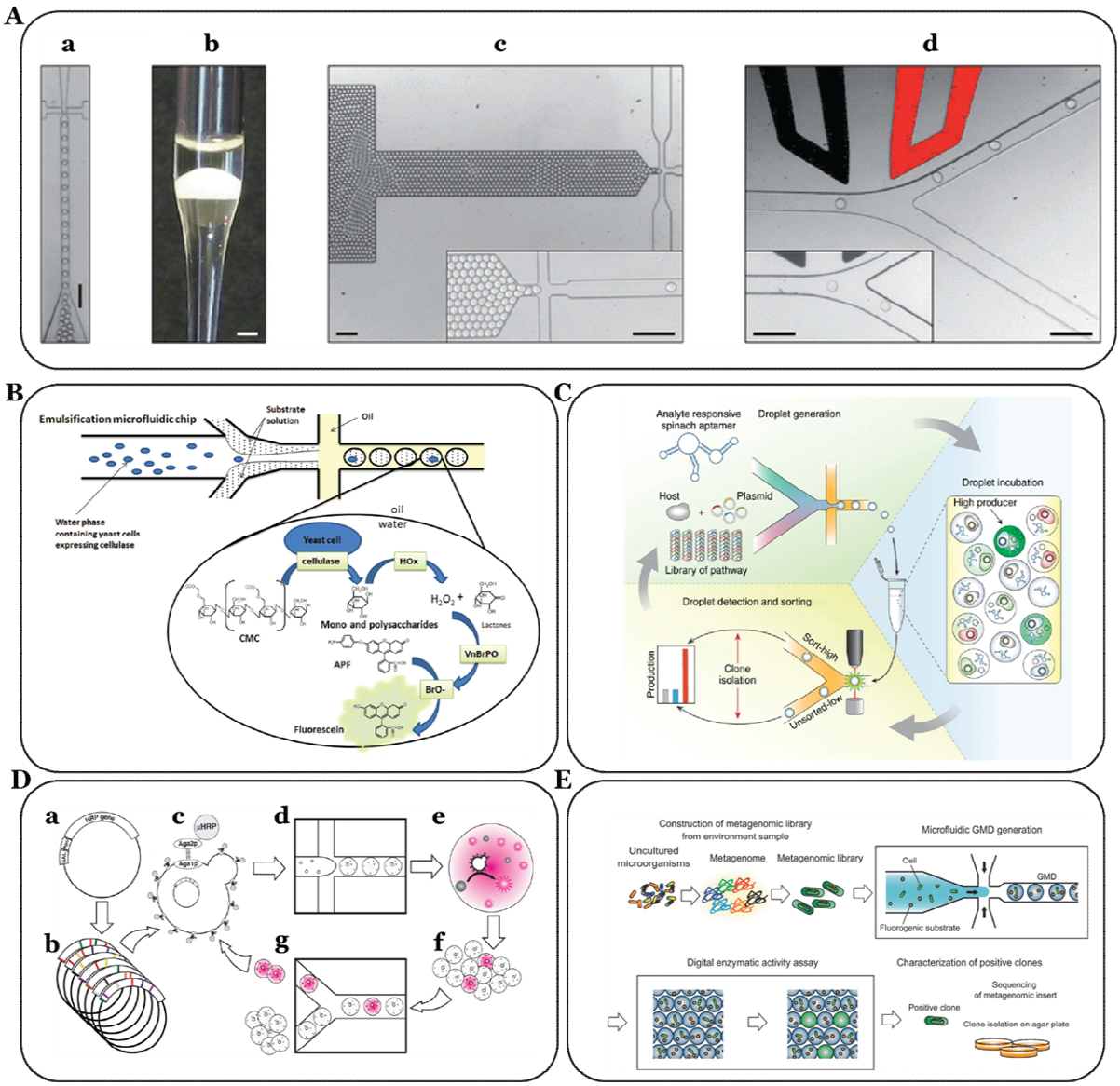

Fig. 7. Phenotype-based single-cell sorting in droplets. (A) A typical experimental protocol of fluorescence-activated droplet sorting (FADS), including (a) droplet generation and cell encapsulation, (b) incubation allowing for phenotype to be tested, (c) re-injection of droplets into sorting device, and (d) phenotype detection by fluorescence sensor and sorting. Scale bars are 100 μm except for (b), where it is 1 mm. (B-C) Different detection routines based on secretory phenotypes. (B) An enzymatic reaction cascade able to convert bait substrates into fluorescein with the help of cellulase expressed by active yeast cells. (C) A RAPID (RNA-aptamers-in-droplets) strategy for sorting phenotypes that are hard to be coupled to fluorescence assays. Fig. 7A to 7C are reprinted from reference[88], [89], and [91] with permissions from (A) the Royal Society of Chemistry 2009, (B) American Institute of Physics 2014, and (C) under a Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/). (D-E) Conceptual schematics of directed evolution and functional metagenomics in droplets. (D) Directed evolution of horseradish peroxidase (HRP) in droplets. (a) Plasmid encoding HRP with an Aga2 gene allowing for surface displace. (b) Mutant library is constructed by error prone PCR (epPCR) and (c) transformed into yeast cells. (d) Single-cell encapsulation with droplet microfluidics. (e-f) Incubation and phenotypic testing in droplets. In this case, active variants convert Amplex UltraRed (AUR, gray) into its fluorescent product (pink). (g)

Droplets are screened by a microfluidics sorter. Brightest droplets are recovered and demulsified, with cells retrieved for another round of screening process. (E) Study of functional metagenomics in droplets. Fig. 7D and 7E are adopted from reference [96] and [99], with permissions from (D) National Academy of Sciences 2017 and (E) Elsevier 2014.

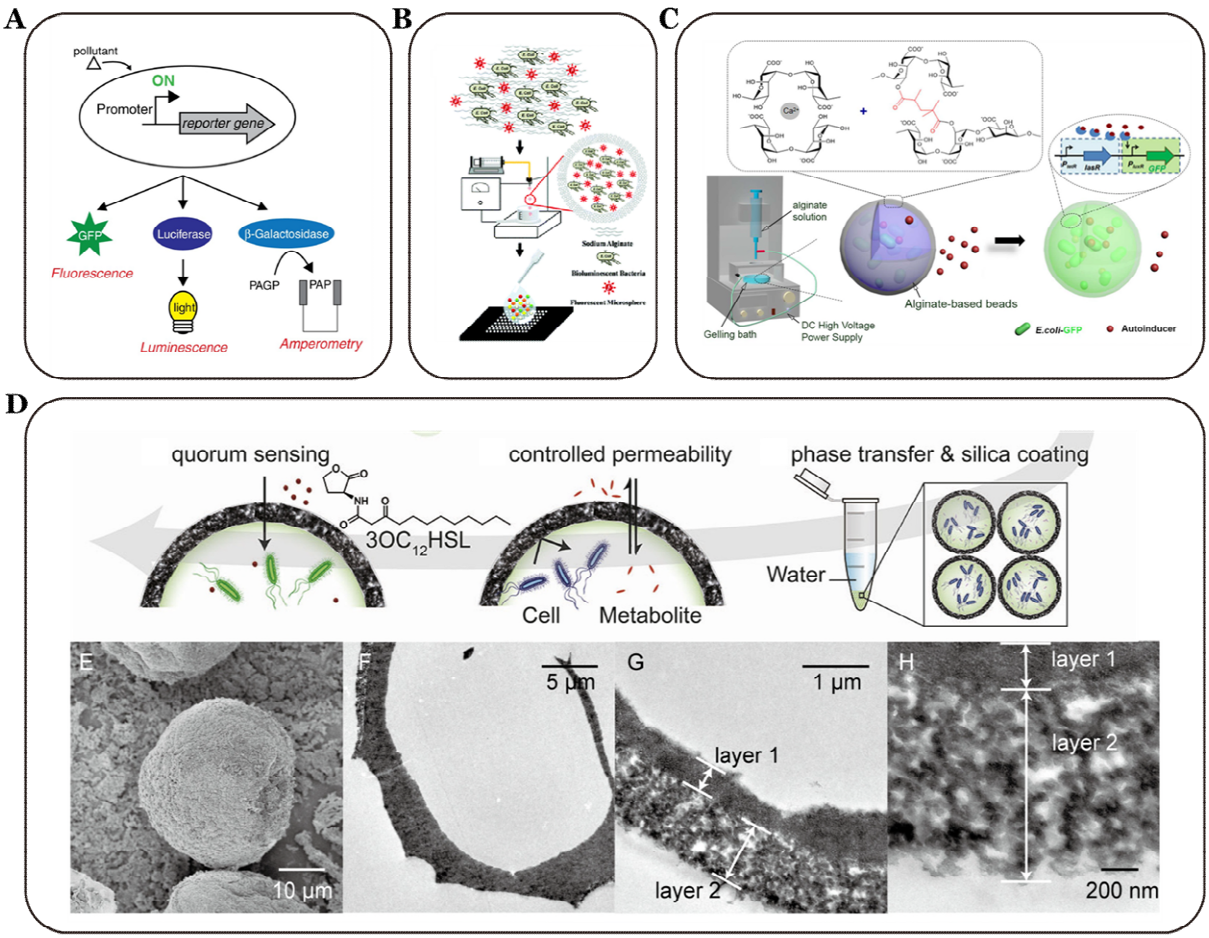

Fig. 8. Living cell biosensors in droplets. (A) Conceptual schematic of bioreporter for environmental monitoring. Genetically engineered microbes are able to convert the target into detectable signals such as fluorescence, bioluminescence, or electrochemistry. (B) A microarray chip capable of immobilizing biosensor-containing alginate microgels. Different fluorophores are used to barcode the microgels. (C-D) Hydrogel enhancement strategies to accommodate bacterial biosensors in gel droplets. (C) Two-step crosslinking method: alginate-MA microbeads are first physically crosslinked by calcium ions and then chemically enhanced by UV radiation. (D) Silica coating method: crosslinked microgels are surface-coated with two layers of silica that prevent cell leakage while controlling the permeability of molecules to microgels. The micrographs are TEM characterizations of the silica coating. Fig. 8A to 8D are adopted from reference [109], [111], [114], and [115], with permissions from (A) and (D) Elsevier 2017 and 2021, respectively, (B) the Royal Society of Chemistry 2010, (C) American Chemical Society 2017.

References

[1] N. Sims, B. Kasprzyk-Hordern, Future perspectives of wastewater-based epidemiology: Monitoring infectious disease spread and resistance to the community level, Environ. Int. 139 (2020) 105689. https://doi.org/10.1016/j.envint.2020.105689.

[2] M. Lorenzo, Y. Picó, Wastewater-based epidemiology: current status and future prospects, Curr. Opin. Environ. Sci. Heal. 9 (2019) 77–84. https://doi.org/10.1016/j.coesh.2019.05.007.

[3] J. Peccia, A. Zulli, D.E. Brackney, N.D. Grubaugh, E.H. Kaplan, A. Casanovas-Massana, A.I. Ko, A.A. Malik, D. Wang, M. Wang, J.L. Warren, D.M. Weinberger, W. Arnold, S.B. Omer, Measurement of SARS-CoV-2 RNA in wastewater tracks community infection dynamics, Nat. Biotechnol. 38 (2020) 1164–1167. https://doi.org/10.1038/s41587-020-0684-z.

[4] Z. Yang, G. Xu, J. Reboud, B. Kasprzyk-Hordern, J.M. Cooper, Monitoring Genetic Population Biomarkers for Wastewater-Based Epidemiology, Anal. Chem. 89 (2017) 9941–9945. https://doi.org/10.1021/acs.analchem.7b02257.

[5] K. Mao, Z. Yang, H. Zhang, X. Li, J.M. Cooper, Paper-based nanosensors to evaluate community-wide illicit drug use for wastewater-based epidemiology, Water Res. 189 (2021) 116559. https://doi.org/10.1016/j.watres.2020.116559.

[6] D. Mao, S. Yu, M. Rysz, Y. Luo, F. Yang, F. Li, J. Hou, Q. Mu, P.J.J. Alvarez, Prevalence and proliferation of antibiotic resistance genes in two municipal wastewater treatment plants, Water Res. 85 (2015) 458–466. https://doi.org/10.1016/j.watres.2015.09.010.

[7] K. Mao, H. Zhang, Y. Pan, Z. Yang, Biosensors for wastewater-based epidemiology for monitoring public health, Water Res. 191 (2020) 116787. https://doi.org/10.1016/j.watres.2020.116787.

[8] S. Rengaraj, Á. Cruz-Izquierdo, J.L. Scott, M. Di Lorenzo, Impedimetric paper-based biosensor for the detection of bacterial contamination in water, Sensors Actuators, B Chem. 265 (2018) 50–58. https://doi.org/10.1016/j.snb.2018.03.020.

[9] R. Gonzalez, K. Curtis, A. Bivins, K. Bibby, M.H. Weir, K. Yetka, H. Thompson, D. Keeling, J. Mitchell, D. Gonzalez, COVID-19 surveillance in Southeastern Virginia using wastewater-based epidemiology, Water Res. 186 (2020) 116296. https://doi.org/10.1016/j.watres.2020.116296.

[10] P.M. D'Aoust, E. Mercier, D. Montpetit, J.J. Jia, I. Alexandrov, N. Neault, A.T. Baig, J. Mayne, X. Zhang, T. Alain, M.A. Langlois, M.R. Servos, M. MacKenzie, D. Figeys, A.E. MacKenzie, T.E. Graber, R. Delatolla, Quantitative analysis of SARS-CoV-2 RNA from wastewater solids in communities with low COVID-19 incidence and prevalence, Water Res. 188 (2021) 116560. https://doi.org/10.1016/j.watres.2020.116560.

[11] W. Ahmed, P.M. Bertsch, N. Angel, K. Bibby, A. Bivins, L. Dierens, J. Edson, J. Ehret, P. Gyawali, K.A. Hamilton, I. Hosegood, P. Hugenholtz, G. Jiang, M. Kitajima, H.T. Sichani, J. Shi, K.M. Shimko, S.L. Simpson, W.J.M. Smith, E.M. Symonds, K. V. Thomas, R. Verhagen, J. Zaugg, J.F. Mueller, Detection of SARS-CoV-2 RNA in commercial passenger aircraft and cruise ship wastewater: a surveillance tool for assessing the presence of COVID-19 infected travellers, J. Travel Med. 27 (2020) 1–11. https://doi.org/10.1093/jtm/taaa116.

[12] F. Lira, I. Vaz-Moreira, J. Tamames, C.M. Manaia, J.L. Martínez, Metagenomic analysis of an

urban resistome before and after wastewater treatment, Sci. Rep. 10 (2020) 1–9. https://doi.org/10.1038/s41598-020-65031-y.

[13] D. Schneider, N. Aßmann, D. Wicke, A. Poehlein, R. Daniel, Metagenomes of Wastewater at Different Treatment Stages in Central Germany, Microbiol. Resour. Announc. 9 (2020) 25–27. https://doi.org/10.1128/mra.00201-20.

[14] R.J. Newton, S.L. McLellan, D.K. Dila, J.H. Vineis, H.G. Morrison, A. Murat Eren, M.L. Sogin, Sewage reflects the microbiomes of human populations, MBio. 6 (2015) 1–9. https://doi.org/10.1128/mBio.02574-14.

[15] D. Numberger, L. Ganzert, L. Zoccarato, K. Mühldorfer, S. Sauer, H.P. Grossart, A.D. Greenwood, Characterization of bacterial communities in wastewater with enhanced taxonomic resolution by full-length 16S rRNA sequencing, Sci. Rep. 9 (2019) 1–14. https://doi.org/10.1038/s41598-019-46015-z.

[16] K. Mao, X. Min, H. Zhang, K. Zhang, H. Cao, Y. Guo, Z. Yang, Paper-based microfluidics for rapid diagnostics and drug delivery, J. Control. Release. 322 (2020) 187–199. https://doi.org/10.1016/j.jconrel.2020.03.010.

[17] Y. Ding, P.D. Howes, A.J. Demello, Recent advances in droplet microfluidics, Anal. Chem. 92 (2020) 132–149. https://doi.org/10.1021/acs.analchem.9b05047.

[18] G.M. Whitesides, The origins and the future of microfluidics, Nature. 442 (2006) 368–373. https://doi.org/10.1038/nature05058.

[19] C.N. Baroud, F. Gallaire, R. Dangla, Dynamics of microfluidic droplets, Lab Chip. 10 (2010) 2032–2045. https://doi.org/10.1039/c001191f.

[20] K. Choi, A.H.C. Ng, R. Fobel, A.R. Wheeler, Digital microfluidics, Annu. Rev. Anal. Chem. 5 (2012) 413–440. https://doi.org/10.1146/annurev-anchem-062011-143028.

[21] X. Hou, Y.S. Zhang, G.T. De Santiago, M.M. Alvarez, J. Ribas, S.J. Jonas, P.S. Weiss, A.M. Andrews, J. Aizenberg, A. Khademhosseini, Interplay between materials and microfluidics, Nat. Rev. Mater. 2 (2017). https://doi.org/10.1038/natrevmats.2017.16.

[22] P. Zhu, L. Wang, Passive and active droplet generation with microfluidics: a review, Lab Chip. 17 (2017) 34–75. https://doi.org/10.1039/C6LC01018K.

[23] B.J. Hindson, K.D. Ness, D.A. Masquelier, P. Belgrader, N.J. Heredia, A.J. Makarewicz, I.J. Bright, M.Y. Lucero, A.L. Hiddessen, T.C. Legler, T.K. Kitano, M.R. Hodel, J.F. Petersen, P.W. Wyatt, E.R. Steenblock, P.H. Shah, L.J. Bousse, C.B. Troup, J.C. Mellen, D.K. Wittmann, N.G. Erndt, T.H. Cauley, R.T. Koehler, A.P. So, S. Dube, K.A. Rose, L. Montesclaros, S. Wang, D.P. Stumbo, S.P. Hodges, S. Romine, F.P. Milanovich, H.E. White, J.F. Regan, G.A. Karlin-Neumann, C.M. Hindson, S. Saxonov, B.W. Colston, High-throughput droplet digital PCR system for absolute quantitation of DNA copy number, Anal. Chem. 83 (2011) 8604–8610. https://doi.org/10.1021/ac202028g.

[24] J.U. Shim, L.F. Olguin, G. Whyte, D. Scott, A. Babtie, C. Abell, W.T.S. Huck, F. Hollfelder, Simultaneous determination of gene expression and enzymatic activity in individual bacterial cells in microdroplet compartments, J. Am. Chem. Soc. 131 (2009) 15251–15256. https://doi.org/10.1021/ja904823z.

[25] J. Kehe, A. Kulesa, A. Ortiz, C.M. Ackerman, S.G. Thakku, D. Sellers, S. Kuehn, J. Gore, J.

Friedman, P.C. Blainey, Massively parallel screening of synthetic microbial communities, Proc. Natl. Acad. Sci. U. S. A. 116 (2019) 12804–12809. https://doi.org/10.1073/pnas.1900102116.

[26] H. Geng, J. Feng, L.M. Stabryla, S.K. Cho, Dielectrowetting manipulation for digital microfluidics: creating, transporting, splitting, and merging of droplets, Lab Chip. 17 (2017) 1060–1068. https://doi.org/10.1039/c7lc00006e.

[27] H.D. Xi, H. Zheng, W. Guo, A.M. Gañán-Calvo, Y. Ai, C.W. Tsao, J. Zhou, W. Li, Y. Huang, N.T. Nguyen, S.H. Tan, Active droplet sorting in microfluidics: a review, Lab Chip. 17 (2017) 751–771. https://doi.org/10.1039/c6lc01435f.

[28] U. Tangen, A. Sharma, P. Wagler, J.S. McCaskill, On demand nanoliter-scale microfluidic droplet generation, injection, and mixing using a passive microfluidic device, Biomicrofluidics. 9 (2015). https://doi.org/10.1063/1.4907895.

[29] E. O'Brien, I. Xagoraraki, A water-focused one-health approach for early detection and prevention of viral outbreaks, One Heal. 7 (2019) 100094. https://doi.org/10.1016/j.onehlt.2019.100094.

[30] Z. Yang, M.A. D'Uriac, S. Goggins, B. Kasprzyk-Hordern, K. V. Thomas, C.G. Frost, P. Estrela, A novel DNA biosensor using a ferrocenyl intercalator applied to the potential detection of human population biomarkers in wastewater, Environ. Sci. Technol. 49 (2015) 5609–5617. https://doi.org/10.1021/acs.est.5b00637.

[31] O.L. Gurvich, M. Skoblov, Real-time PCR and multiplex approaches, Methods Mol. Biol. 784 (2011) 1–13. https://doi.org/10.1007/978-1-61779-289-2_1.

[32] K.R. Sreejith, C.H. Ooi, J. Jin, D.V. Dao, N.T. Nguyen, Digital polymerase chain reaction technology-recent advances and future perspectives, Lab Chip. 18 (2018) 3717–3732. https://doi.org/10.1039/c8lc00990b.

[33] N. Rački, T. Dreo, I. Gutierrez-Aguirre, A. Blejec, M. Ravnikar, Reverse transcriptase droplet digital PCR shows high resilience to PCR inhibitors from plant, soil and water samples, Plant Methods. 10 (2014) 1–10. https://doi.org/10.1186/s13007-014-0042-6.

[34] L.B. Pinheiro, V.A. Coleman, C.M. Hindson, J. Herrmann, B.J. Hindson, S. Bhat, K.R. Emslie, Evaluation of a droplet digital polymerase chain reaction format for DNA copy number quantification, Anal. Chem. 84 (2012) 1003–1011. https://doi.org/10.1021/ac202578x.

[35] H. Li, R. Bai, Z. Zhao, L. Tao, M. Ma, Z. Ji, M. Jian, Z. Ding, X. Dai, F. Bao, A. Liu, Application of droplet digital PCR to detect the pathogens of infectious diseases, Biosci. Rep. 38 (2018) 1–8. https://doi.org/10.1042/BSR20181170.

[36] T.J. Abram, H. Cherukury, C.Y. Ou, T. Vu, M. Toledano, Y. Li, J.T. Grunwald, M.N. Toosky, D.F. Tifrea, A. Slepenkin, J. Chong, L. Kong, D.V. Del Pozo, K.T. La, L. Labanieh, J. Zimak, B. Shen, S.S. Huang, E. Gratton, E.M. Peterson, W. Zhao, Rapid bacterial detection and antibiotic susceptibility testing in whole blood using one-step, high throughput blood digital PCR, Lab Chip. 20 (2020) 477–489. https://doi.org/10.1039/c9lc01212e.

[37] G. Gobert, A. Cotillard, C. Fourmestraux, L. Pruvost, J. Miguet, M. Boyer, Droplet digital PCR improves absolute quantification of viable lactic acid bacteria in faecal samples, J. Microbiol. Methods. 148 (2018) 64–73. https://doi.org/https://doi.org/10.1016/j.mimet.2018.03.004.

[38] E. Capo, G. Spong, S. Koizumi, I. Puts, F. Olajos, H. Königsson, J. Karlsson, P. Byström,

Droplet digital PCR applied to environmental DNA, a promising method to estimate fish population abundance from humic-rich aquatic ecosystems, Environ. DNA. (2020) 1–10. https://doi.org/10.1002/edn3.115.

[39] M.A. Jahne, N.E. Brinkman, S.P. Keely, B.D. Zimmerman, E.A. Wheaton, J.L. Garland, Droplet digital PCR quantification of norovirus and adenovirus in decentralized wastewater and graywater collections: Implications for onsite reuse, Water Res. 169 (2020) 115213. https://doi.org/10.1016/j.watres.2019.115213.

[40] M.A. Jahne, M.E. Schoen, J.L. Garland, N.J. Ashbolt, Simulation of enteric pathogen concentrations in locally-collected greywater and wastewater for microbial risk assessments, Microb. Risk Anal. 5 (2017) 44–52. https://doi.org/10.1016/j.mran.2016.11.001.

[41] T. Suo, X. Liu, J. Feng, M. Guo, W. Hu, D. Guo, H. Ullah, Y. Yang, Q. Zhang, X. Wang, M. Sajid, Z. Huang, L. Deng, T. Chen, F. Liu, K. Xu, Y. Liu, Q. Zhang, Y. Liu, Y. Xiong, G. Chen, K. Lan, Y. Chen, ddPCR: a more accurate tool for SARS-CoV-2 detection in low viral load specimens, Emerg. Microbes Infect. 9 (2020) 1259–1268. https://doi.org/10.1080/22221751.2020.1772678.

[42] M. Pfammatter, M. Andreasen, G. Meisl, C.G. Taylor, J. Adamcik, S. Bolisetty, A. Sánchez-Ferrer, D. Klenerman, C.M. Dobson, R. Mezzenga, T.P.J. Knowles, A. Aguzzi, S. Hornemann, Absolute Quantification of Amyloid Propagons by Digital Microfluidics, Anal. Chem. 89 (2017) 12306–12313. https://doi.org/10.1021/acs.analchem.7b03279.

[43] T.P.J. Knowles, M. Vendruscolo, C.M. Dobson, The amyloid state and its association with protein misfolding diseases, Nat. Rev. Mol. Cell Biol. 15 (2014) 384–396. https://doi.org/10.1038/nrm3810.

[44] I. Morales-Belpaire, P.A. Gerin, Fate of amyloid fibrils introduced in wastewater sludge, Water Res. 42 (2008) 4449–4456. https://doi.org/10.1016/j.watres.2008.07.034.

[45] D.J. Collins, A. Neild, A. deMello, A.Q. Liu, Y. Ai, The Poisson distribution and beyond: Methods for microfluidic droplet production and single cell encapsulation, Lab Chip. 15 (2015) 3439–3459. https://doi.org/10.1039/c5lc00614g.

[46] L. Mazutis, J. Gilbert, W.L. Ung, D.A. Weitz, A.D. Griffiths, J.A. Heyman, Single-cell analysis and sorting using droplet-based microfluidics, Nat. Protoc. 8 (2013) 870–891. https://doi.org/10.1038/nprot.2013.046.

[47] J.F. Edd, D. Di Carlo, K.J. Humphry, S. Köster, D. Irimia, D.A. Weitz, M. Toner, Controlled encapsulation of single-cells into monodisperse picolitre drops, Lab Chip. 8 (2008) 1262–1264. https://doi.org/10.1039/b805456h.

[48] A.S. Mao, J.W. Shin, S. Utech, H. Wang, O. Uzun, W. Li, M. Cooper, Y. Hu, L. Zhang, D.A. Weitz, D.J. Mooney, Deterministic encapsulation of single cells in thin tunable microgels for niche modelling and therapeutic delivery, Nat. Mater. 16 (2017) 236–243. https://doi.org/10.1038/nmat4781.

[49] N. Pacocha, O. Scheler, M.M. Nowak, L. Derzsi, J. Cichy, P. Garstecki, Direct droplet digital PCR (dddPCR) for species specific, accurate and precise quantification of bacteria in mixed samples, Anal. Methods. 11 (2019) 5730–5735. https://doi.org/10.1039/c9ay01874c.

[50] X. Xu, J. Wang, L. Wu, J. Guo, Y. Song, T. Tian, W. Wang, Z. Zhu, C. Yang, Microfluidic Single-

Cell Omics Analysis, Small. 16 (2020) 1–17. https://doi.org/10.1002/smll.201903905.

[51] A. Rakszewska, R.J. Stolper, A.B. Kolasa, A. Piruska, W.T.S. Huck, Quantitative Single-Cell mRNA Analysis in Hydrogel Beads, Angew. Chemie - Int. Ed. 55 (2016) 6698–6701. https://doi.org/10.1002/anie.201601969.

[52] S. Guo, W.N. Lin, Y. Hu, G. Sun, D.T. Phan, C.H. Chen, Ultrahigh-throughput droplet microfluidic device for single-cell miRNA detection with isothermal amplification, Lab Chip. 18 (2018) 1914–1920. https://doi.org/10.1039/c8lc00390d.

[53] T.D. Rane, H.C. Zec, C. Puleo, A.P. Lee, T.H. Wang, Droplet microfluidics for amplification-free genetic detection of single cells, Lab Chip. 12 (2012) 3341–3347. https://doi.org/10.1039/c2lc40537g.

[54] K.E. Mach, A.M. Kaushik, K. Hsieh, P.K. Wong, T.H. Wang, J.C. Liao, Optimizing peptide nucleic acid probes for hybridization-based detection and identification of bacterial pathogens, Analyst. 144 (2019) 1565–1574. https://doi.org/10.1039/c8an02194e.

[55] M. Xiao, K. Zou, L. Li, L. Wang, Y. Tian, C. Fan, H. Pei, Stochastic DNA Walkers in Droplets for Super-Multiplexed Bacterial Phenotype Detection, Angew. Chemie. 131 (2019) 15594–15600. https://doi.org/10.1002/ange.201906438.

[56] D.K. Kang, M.M. Ali, K. Zhang, S.S. Huang, E. Peterson, M.A. Digman, E. Gratton, W. Zhao, Rapid detection of single bacteria in unprocessed blood using Integrated Comprehensive Droplet Digital Detection, Nat. Commun. 5 (2014) 1–10. https://doi.org/10.1038/ncomms6427.

[57] M.M. Ali, S.D. Aguirre, H. Lazim, Y. Li, Fluorogenic DNAzyme probes as bacterial indicators, Angew. Chemie - Int. Ed. 50 (2011) 3751–3754. https://doi.org/10.1002/anie.201100477.

[58] F. Lyu, M. Xu, Y. Cheng, J. Xie, J. Rao, S.K.Y. Tang, Quantitative detection of cells expressing BlaC using droplet-based microfluidics for use in the diagnosis of tuberculosis, Biomicrofluidics. 9 (2015) 1–9. https://doi.org/10.1063/1.4928879.

[59] X. Cui, L. Ren, Y. Shan, X. Wang, Z. Yang, C. Li, J. Xu, B. Ma, Smartphone-based rapid quantification of viable bacteria by single-cell microdroplet turbidity imaging, Analyst. 143 (2018) 3309–3316. https://doi.org/10.1039/c8an00456k.

[60] Q. Zhang, S. Savagatrup, P. Kaplonek, P.H. Seeberger, T.M. Swager, Janus Emulsions for the Detection of Bacteria, ACS Cent. Sci. 3 (2017) 309–313. https://doi.org/10.1021/acscentsci.7b00021.

[61] L. Zeininger, S. Nagelberg, K.S. Harvey, S. Savagatrup, M.B. Herbert, K. Yoshinaga, J.A. Capobianco, M. Kolle, T.M. Swager, Rapid Detection of Salmonella enterica via Directional Emission from Carbohydrate-Functionalized Dynamic Double Emulsions, ACS Cent. Sci. 5 (2019) 789–795. https://doi.org/10.1021/acscentsci.9b00059.

[62] T. Woyke, D.F.R. Doud, F. Schulz, The trajectory of microbial single-cell sequencing, Nat. Methods. 14 (2017) 1045–1054. https://doi.org/10.1038/nmeth.4469.

[63] A.E.K. Dichosa, A.R. Daughton, K.G. Reitenga, M.S. Fitzsimons, C.S. Han, Capturing and cultivating single bacterial cells in gel microdroplets to obtain near-complete genomes, Nat. Protoc. 9 (2014) 608–621. https://doi.org/10.1038/nprot.2014.034.

[64] M.S. Fitzsimons, M. Novotny, C.C. Lo, A.E.K. Dichosa, J.L. Yee-Greenbaum, J.P. Snook, W.

Gu, O. Chertkov, K.W. Davenport, K. McMurry, K.G. Reitenga, A.R. Daughton, J. He, S.L. Johnson, C.D. Gleasner, P.L. Wills, B. Parson-Quintana, P.S. Chain, J.C. Detter, R.S. Lasken, C.S. Han, Nearly finished genomes produced using gel microdroplet culturing reveal substantial intraspecies genomic diversity within the human microbiome, Genome Res. 23 (2013) 878–888. https://doi.org/10.1101/gr.142208.112.

[65] M. Hosokawa, Y. Nishikawa, M. Kogawa, H. Takeyama, Massively parallel whole genome amplification for single-cell sequencing using droplet microfluidics, Sci. Rep. 7 (2017) 3–4. https://doi.org/10.1038/s41598-017-05436-4.

[66] F. Lan, B. Demaree, N. Ahmed, A.R. Abate, Single-cell genome sequencing at ultra-high-throughput with microfluidic droplet barcoding, Nat. Biotechnol. 35 (2017) 640–646. https://doi.org/10.1038/nbt.3880.

[67] Y. Fu, C. Li, S. Lu, W. Zhou, F. Tang, X.S. Xie, Y. Huang, Uniform and accurate single-cell sequencing based on emulsion whole-genome amplification, Proc. Natl. Acad. Sci. U. S. A. 112 (2015) 11923–11928. https://doi.org/10.1073/pnas.1513988112.

[68] D. Thibault, P.A. Jensen, S. Wood, C. Qabar, S. Clark, M.G. Shainheit, R.R. Isberg, T. van Opijnen, Droplet Tn-Seq combines microfluidics with Tn-Seq for identifying complex single-cell phenotypes, Nat. Commun. 10 (2019). https://doi.org/10.1038/s41467-019-13719-9.

[69] S.J. Spencer, M. V. Tamminen, S.P. Preheim, M.T. Guo, A.W. Briggs, I.L. Brito, D. A Weitz, L.K. Pitkänen, F. Vigneault, M.P. Virta, E.J. Alm, Massively parallel sequencing of single cells by epicPCR links functional genes with phylogenetic markers, ISME J. 10 (2016) 427–436. https://doi.org/10.1038/ismej.2015.124.

[70] E.Z. Macosko, A. Basu, R. Satija, J. Nemesh, K. Shekhar, M. Goldman, I. Tirosh, A.R. Bialas, N. Kamitaki, E.M. Martersteck, J.J. Trombetta, D.A. Weitz, J.R. Sanes, A.K. Shalek, A. Regev, S.A. McCarroll, Highly parallel genome-wide expression profiling of individual cells using nanoliter droplets, Cell. 161 (2015) 1202–1214. https://doi.org/10.1016/j.cell.2015.05.002.

[71] A.M. Klein, L. Mazutis, I. Akartuna, N. Tallapragada, A. Veres, V. Li, L. Peshkin, D.A. Weitz, M.W. Kirschner, Droplet barcoding for single-cell transcriptomics applied to embryonic stem cells, Cell. 161 (2015) 1187–1201. https://doi.org/10.1016/j.cell.2015.04.044.

[72] N. Schaum, J. Karkanias, N.F. Neff, A.P. May, S.R. Quake, T. Wyss-Coray, S. Darmanis, J. Batson, O. Botvinnik, M.B. Chen, S. Chen, F. Green, R.C. Jones, A. Maynard, L. Penland, A.O. Pisco, R. V. Sit, G.M. Stanley, J.T. Webber, F. Zanini, A.S. Baghel, I. Bakerman, I. Bansal, D. Berdnik, B. Bilen, D. Brownfield, C. Cain, M. Cho, G. Cirolia, S.D. Conley, A. Demers, K. Demir, A. de Morree, T. Divita, H. du Bois, L.B.T. Dulgeroff, H. Ebadi, F.H. Espinoza, M. Fish, Q. Gan, B.M. George, A. Gillich, G. Genetiano, X. Gu, G.S. Gulati, Y. Hang, S. Hosseinzadeh, A. Huang, T. Iram, T. Isobe, F. Ives, K.S. Kao, G. Karnam, A.M. Kershner, B.M. Kiss, W. Kong, M.E. Kumar, J.Y. Lam, D.P. Lee, S.E. Lee, G. Li, Q. Li, L. Liu, A. Lo, W.J. Lu, A. Manjunath, K.L. May, O.L. May, M. McKay, R.J. Metzger, M. Mignardi, D. Min, A.N. Nabhan, K.M. Ng, J. Noh, R. Patkar, W.C. Peng, R. Puccinelli, E.J. Rulifson, S.S. Sikandar, R. Sinha, K. Szade, W. Tan, C. Tato, K. Tellez, K.J. Travaglini, C. Tropini, L. Waldburger, L.J. van Weele, M.N. Wosczyna, J. Xiang, S. Xue, J. Youngyunpipatkul, M.E. Zardeneta, F. Zhang, L. Zhou, P. Castro, D. Croote, J.L. DeRisi, C.S. Kuo, B. Lehallier, P.K. Nguyen, S.Y. Tan, B.M. Wang, H. Yousef, P.A. Beachy,

C.K.F. Chan, K.C. Huang, K. Weinberg, S.M. Wu, B.A. Barres, M.F. Clarke, S.K. Kim, M.A. Krasnow, R. Nusse, T.A. Rando, J. Sonnenburg, I.L. Weissman, Single-cell transcriptomics of 20 mouse organs creates a Tabula Muris, Nature. 562 (2018) 367–372. https://doi.org/10.1038/s41586-018-0590-4.

[73] J.A. Briggs, C. Weinreb, D.E. Wagner, S. Megason, L. Peshkin, M.W. Kirschner, A.M. Klein, The dynamics of gene expression in vertebrate embryogenesis at single-cell resolution, Science (80-.). 360 (2018). https://doi.org/10.1126/science.aar5780.

[74] D.E. Wagner, C. Weinreb, Z.M. Collins, J.A. Briggs, S.G. Megason, A.M. Klein, Single-cell mapping of gene expression landscapes and lineage in the zebrafish embryo, Science (80-.). 360 (2018) 981–987. https://doi.org/10.1126/science.aar4362.

[75] J.A. Farrell, Y. Wang, S.J. Riesenfeld, K. Shekhar, A. Regev, A.F. Schier, Single-cell reconstruction of developmental trajectories during zebrafish embryogenesis, Science (80-.). 360 (2018). https://doi.org/10.1126/science.aar3131.

[76] H. Tekin, S. Simmons, B. Cummings, L. Gao, X. Adiconis, C.C. Hession, A. Ghoshal, D. Dionne, S.R. Choudhury, V. Yesilyurt, N.E. Sanjana, X. Shi, C. Lu, M. Heidenreich, J.Q. Pan, J.Z. Levin, F. Zhang, Effects of 3D culturing conditions on the transcriptomic profile of stem-cell-derived neurons, Nat. Biomed. Eng. 2 (2018) 540–554. https://doi.org/10.1038/s41551-018-0219-9.

[77] B. Adamson, T.M. Norman, M. Jost, M.Y. Cho, J.K. Nuñez, Y. Chen, J.E. Villalta, L.A. Gilbert, M.A. Horlbeck, M.Y. Hein, R.A. Pak, A.N. Gray, C.A. Gross, A. Dixit, O. Parnas, A. Regev, J.S. Weissman, A Multiplexed Single-Cell CRISPR Screening Platform Enables Systematic Dissection of the Unfolded Protein Response, Cell. 167 (2016) 1867-1882.e21. https://doi.org/10.1016/j.cell.2016.11.048.

[78] Y. Zhang, J. Gao, Y. Huang, J. Wang, Recent Developments in Single-Cell RNA-Seq of Microorganisms, Biophys. J. 115 (2018) 173–180. https://doi.org/10.1016/j.bpj.2018.06.008.

[79] A. Kuchina, L.M. Brettner, L. Paleologu, C.M. Roco, A.B. Rosenberg, A. Carignano, R. Kibler, M. Hirano, R.W. DePaolo, G. Seelig, Microbial single-cell RNA sequencing by split-pool barcoding, Science (80-.). 371 (2021) eaba5257. https://doi.org/10.1126/science.aba5257.

[80] F. Imdahl, E. Vafadarnejad, C. Homberger, A.E. Saliba, J. Vogel, Single-cell RNA-sequencing reports growth-condition-specific global transcriptomes of individual bacteria, Nat. Microbiol. 5 (2020) 1202–1206. https://doi.org/10.1038/s41564-020-0774-1.

[81] K. Sheng, W. Cao, Y. Niu, Q. Deng, C. Zong, Effective detection of variation in single-cell transcriptomes using MATQ-seq, Nat. Methods. 14 (2017) 267–270. https://doi.org/10.1038/nmeth.4145.

[82] L. Liu, C.K. Dalal, B.M. Heineike, A.R. Abate, High throughput gene expression profiling of yeast colonies with microgel-culture Drop-seq, Lab Chip. 19 (2019) 1838–1849. https://doi.org/10.1039/c9lc00084d.

[83] P.Y. Colin, B. Kintses, F. Gielen, C.M. Miton, G. Fischer, M.F. Mohamed, M. Hyvönen, D.P. Morgavi, D.B. Janssen, F. Hollfelder, Ultrahigh-throughput discovery of promiscuous enzymes by picodroplet functional metagenomics, Nat. Commun. 6 (2015). https://doi.org/10.1038/ncomms10008.

[84] P.S. Dittrich, A. Manz, Lab-on-a-chip: Microfluidics in drug discovery, Nat. Rev. Drug Discov.

5 (2006) 210–218. https://doi.org/10.1038/nrd1985.

[85] P. Neužil, S. Giselbrecht, K. Länge, T.J. Huang, A. Manz, Revisiting lab-on-a-chip technology for drug discovery, Nat. Rev. Drug Discov. 11 (2012) 620–632. https://doi.org/10.1038/nrd3799.

[86] A. Kulesa, J. Kehe, J.E. Hurtado, P. Tawde, P.C. Blainey, Combinatorial drug discovery in nanoliter droplets, Proc. Natl. Acad. Sci. U. S. A. 115 (2018). https://doi.org/10.1073/pnas.1802233115.

[87] M. Sivaramakrishnan, R. Kothandan, D.K. Govindarajan, Y. Meganathan, K. Kandaswamy, Active microfluidic systems for cell sorting and separation, Curr. Opin. Biomed. Eng. 13 (2020) 60–68. https://doi.org/10.1016/j.cobme.2019.09.014.

[88] J.C. Baret, O.J. Miller, V. Taly, M. Ryckelynck, A. El-Harrak, L. Frenz, C. Rick, M.L. Samuels, J.B. Hutchison, J.J. Agresti, D.R. Link, D.A. Weitz, A.D. Griffiths, Fluorescence-activated droplet sorting (FADS): Efficient microfluidic cell sorting based on enzymatic activity, Lab Chip. 9 (2009) 1850–1858. https://doi.org/10.1039/b902504a.

[89] R. Ostafe, R. Prodanovic, W.L. Ung, D.A. Weitz, R. Fischer, A high-throughput cellulase screening system based on droplet microfluidics, Biomicrofluidics. 8 (2014) 6–10. https://doi.org/10.1063/1.4886771.

[90] B.L. Wang, A. Ghaderi, H. Zhou, J. Agresti, D.A. Weitz, G.R. Fink, G. Stephanopoulos, Microfluidic high-throughput culturing of single cells for selection based on extracellular metabolite production or consumption, Nat. Biotechnol. 32 (2014) 473–478. https://doi.org/10.1038/nbt.2857.

[91] J. Abatemarco, M.F. Sarhan, J.M. Wagner, J.L. Lin, L. Liu, W. Hassouneh, S.F. Yuan, H.S. Alper, A.R. Abate, RNA-aptamers-in-droplets (RAPID) high-throughput screening for secretory phenotypes, Nat. Commun. 8 (2017). https://doi.org/10.1038/s41467-017-00425-7.

[92] R.J. Best, J.J. Lyczakowski, S. Abalde-Cela, Z. Yu, C. Abell, A.G. Smith, Label-Free Analysis and Sorting of Microalgae and Cyanobacteria in Microdroplets by Intrinsic Chlorophyll Fluorescence for the Identification of Fast Growing Strains, Anal. Chem. 88 (2016) 10445–10451. https://doi.org/10.1021/acs.analchem.6b02364.

[93] Z. Yu, C.R. Boehm, J.M. Hibberd, C. Abell, J. Haseloff, S.J. Burgess, I. Reyna-Llorens, Droplet-based microfluidic analysis and screening of single plant cells, PLoS One. 13 (2018). https://doi.org/10.1371/journal.pone.0196810.

[94] X. Wang, Y. Xin, L. Ren, Z. Sun, P. Zhu, Y. Ji, C. Li, J. Xu, B. Ma, Positive dielectrophoresis-based Raman-activated droplet sorting for culture-free and label-free screening of enzyme function in vivo, Sci. Adv. 6 (2020). https://doi.org/10.1126/sciadv.abb3521.

[95] F. Gielen, R. Hours, S. Emond, M. Fischlechner, U. Schell, F. Hollfelder, Ultrahigh-throughput-directed enzyme evolution by absorbance-activated droplet sorting (AADS), Proc. Natl. Acad. Sci. U. S. A. 113 (2016) E7383–E7389. https://doi.org/10.1073/pnas.1606927113.

[96] J.J. Agresti, E. Antipov, A.R. Abate, K. Ahn, A.C. Rowat, J.C. Baret, M. Marquez, A.M. Klibanov, A.D. Griffiths, D.A. Weitz, Ultrahigh-throughput screening in drop-based microfluidics for directed evolution, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 4004–4009. https://doi.org/10.1073/pnas.0910781107.

[97] P. Mair, F. Gielen, F. Hollfelder, Exploring sequence space in search of functional enzymes using microfluidic droplets, Curr. Opin. Chem. Biol. 37 (2017) 137–144. https://doi.org/10.1016/j.cbpa.2017.02.018.

[98] H.A. Bunzel, X. Garrabou, M. Pott, D. Hilvert, Speeding up enzyme discovery and engineering with ultrahigh-throughput methods, Curr. Opin. Struct. Biol. 48 (2018) 149–156. https://doi.org/10.1016/j.sbi.2017.12.010.

[99] M. Hosokawa, Y. Hoshino, Y. Nishikawa, T. Hirose, D.H. Yoon, T. Mori, T. Sekiguchi, S. Shoji, H. Takeyama, Droplet-based microfluidics for high-throughput screening of a metagenomic library for isolation of microbial enzymes, Biosens. Bioelectron. 67 (2015) 379–385. https://doi.org/10.1016/j.bios.2014.08.059.

[100] M. Najah, R. Calbrix, I.P. Mahendra-Wijaya, T. Beneyton, A.D. Griffiths, A. Drevelle, Droplet-based microfluidics platform for ultra-high-throughput bioprospecting of cellulolytic microorganisms, Chem. Biol. 21 (2014) 1722–1732. https://doi.org/10.1016/j.chembiol.2014.10.020.

[101] J.Q. Boedicker, L. Li, T.R. Kline, R.F. Ismagilov, Detecting bacteria and determining their susceptibility to antibiotics by stochastic confinement in nanoliter droplets using plug-based microfluidics, Lab Chip. 8 (2008) 1265–1272. https://doi.org/10.1039/b804911d.

[102] A.M. Kaushik, K. Hsieh, L. Chen, D.J. Shin, J.C. Liao, T.H. Wang, Accelerating bacterial growth detection and antimicrobial susceptibility assessment in integrated picoliter droplet platform, Biosens. Bioelectron. 97 (2017) 260–266. https://doi.org/10.1016/j.bios.2017.06.006.

[103] Y.J. Eun, A.S. Utada, M.F. Copeland, S. Takeuchi, D.B. Weibel, Encapsulating bacteria in agarose microparticles using microfluidics for high-throughput cell analysis and isolation, ACS Chem. Biol. 6 (2011) 260–266. https://doi.org/10.1021/cb100336p.

[104] S.S. Terekhov, I. V. Smirnov, M. V. Malakhova, A.E. Samoilov, A.I. Manolo, A.S. Nazarov, D. V. Danilov, S.A. Dubiley, I.A. Osterman, M.P. Rubtsova, E.S. Kostryukova, R.H. Ziganshin, M.A. Kornienko, A.A. Vanyushkina, O.N. Bukato, E.N. Ilina, V. V. Vlasov, K. V. Severinov, A.G. Gabibov, S. Altmani, Ultrahigh-throughput functional profiling of microbiota communities, Proc. Natl. Acad. Sci. U. S. A. 115 (2018) 9551–9556. https://doi.org/10.1073/pnas.1811250115.

[105] X. Liu, R.E. Painter, K. Enesa, D. Holmes, G. Whyte, C.G. Garlisi, F.J. Monsma, M. Rehak, F.F. Craig, C.A. Smith, High-throughput screening of antibiotic-resistant bacteria in picodroplets, Lab Chip. 16 (2016) 1636–1643. https://doi.org/10.1039/c6lc00180g.

[106] I. Sinn, P. Kinnunen, T. Albertson, B.H. McNaughton, D.W. Newton, M.A. Burns, R. Kopelman, Asynchronous magnetic bead rotation (AMBR) biosensor in microfluidic droplets for rapid bacterial growth and susceptibility measurements, Lab Chip. 11 (2011) 2604–2611. https://doi.org/10.1039/c0lc00734j.

[107] R. Pol, F. Céspedes, D. Gabriel, M. Baeza, Microfluidic lab-on-a-chip platforms for environmental monitoring, TrAC - Trends Anal. Chem. 95 (2017) 62–68. https://doi.org/10.1016/j.trac.2017.08.001.

[108] H. Harms, M.C. Wells, J.R. Van Der Meer, Whole-cell living biosensors - Are they ready for environmental application?, Appl. Microbiol. Biotechnol. 70 (2006) 273–280.

https://doi.org/10.1007/s00253-006-0319-4.

[109] C. Roggo, J.R. van der Meer, Miniaturized and integrated whole cell living bacterial sensors in field applicable autonomous devices, Curr. Opin. Biotechnol. 45 (2017) 24–33. https://doi.org/10.1016/j.copbio.2016.11.023.

[110] N. Buffi, D. Merulla, J. Beutier, F. Barbaud, S. Beggah, H. Van Lintel, P. Renaud, J. Roelof Van Der Meer, Development of a microfluidics biosensor for agarose-bead immobilized Escherichia coli bioreporter cells for arsenite detection in aqueous samples, Lab Chip. 11 (2011) 2369–2377. https://doi.org/10.1039/c1lc20274j.

[111] J.M. Ahn, J.H. Kim, J.H. Kim, M.B. Gu, Randomly distributed arrays of optically coded functional microbeads for toxicity screening and monitoring, Lab Chip. 10 (2010) 2695–2701. https://doi.org/10.1039/c004942e.

[112] A. Meyer, R. Pellaux, S. Potot, K. Becker, H.P. Hohmann, S. Panke, M. Held, Optimization of a whole-cell biocatalyst by employing genetically encoded product sensors inside nanolitre reactors, Nat. Chem. 7 (2015) 673–678. https://doi.org/10.1038/nchem.2301.

[113] L. Mahler, K. Wink, R.J. Beulig, K. Scherlach, M. Tovar, E. Zang, K. Martin, C. Hertweck, D. Belder, M. Roth, Detection of antibiotics synthetized in microfluidic picolitre-droplets by various actinobacteria, Sci. Rep. 8 (2018) 1–11. https://doi.org/10.1038/s41598-018-31263-2.

[114] P. Li, M. Müller, M.W. Chang, M. Frettlöh, H. Schönherr, Encapsulation of Autoinducer Sensing Reporter Bacteria in Reinforced Alginate-Based Microbeads, ACS Appl. Mater. Interfaces. 9 (2017) 22321–22331. https://doi.org/10.1021/acsami.7b07166.

[115] S. Zhao, H. Wen, Y. Ou, M. Li, L. Wang, H. Zhou, B. Di, Z. Yu, C. Hu, A new design for living cell-based biosensors: Microgels with a selectively permeable shell that can harbor bacterial species, Sensors Actuators, B Chem. 334 (2021) 129648. https://doi.org/10.1016/j.snb.2021.129648.

[116] C.G. Daughton, Monitoring wastewater for assessing community health: Sewage Chemical-Information Mining (SCIM), Sci. Total Environ. 619–620 (2018) 748–764. https://doi.org/10.1016/j.scitotenv.2017.11.102.

[117] C. Chen, C. Kostakis, J.P. Gerber, B.J. Tscharke, R.J. Irvine, J.M. White, Towards finding a population biomarker for wastewater epidemiology studies, Sci. Total Environ. 487 (2014) 621–628. https://doi.org/10.1016/j.scitotenv.2013.11.075.

[118] C. Zhao, Z. Ge, C. Yang, Microfluidic Techniques for Analytes Concentration, Micromachines. 8 (2017) 28. https://doi.org/10.3390/mi8010028.

[119] A.K. Balasubramanian, K.A. Soni, A. Beskok, S.D. Pillai, A microfluidic device for continuous capture and concentration of microorganisms from potable water, Lab Chip. 7 (2007) 1315–1321. https://doi.org/10.1039/b706559k.

[120] M. Yew, Y. Ren, K.S. Koh, C. Sun, C. Snape, A Review of State-of-the-Art Microfluidic Technologies for Environmental Applications: Detection and Remediation, Glob. Challenges. 3 (2019) 1800060. https://doi.org/10.1002/gch2.201800060.