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Leveraging interactions in microfluidic droplets for enhanced biotechnology screens

Carolus Vitalis and Tobias Wenzel

Microfluidic droplet screens serve as an innovative platform for high-throughput biotechnology, enabling significant advancements in discovery, product optimization, and analysis. This review sheds light on the emerging trends of interaction assays in microfluidic droplets, underscoring the unique suitability of droplets for these applications. Encompassing a diverse range of biological entities such as antibodies, enzymes, DNA, RNA, various microbial and mammalian cell types, drugs, and other molecules, these assays demonstrate their versatility and scope. Recent methodological breakthroughs have escalated these screens to novel scales of bioanalysis and biotechnological product design. Moreover, we highlight pioneering advancements that extend droplet-based screens into new domains: cargo delivery within human bodies, application of synthetic gene circuits in natural environments, 3D printing, and the development of droplet structures responsive to environmental signals. The potential of this field is profound and only set to increase.

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The power of interaction screens in droplets

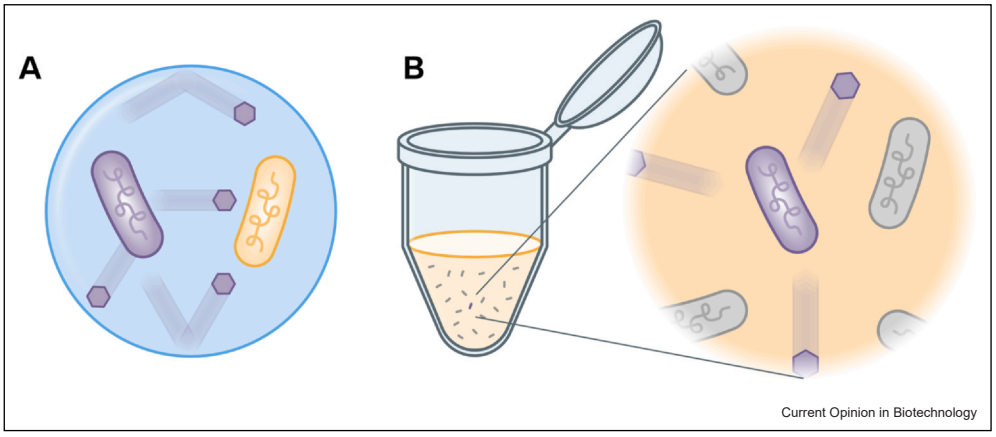
In the rapidly advancing field of biotechnology, interaction screens in droplets are emerging as an increasingly versatile and powerful tool. Microfluidic droplets stand out for their ability to facilitate strong and rapid interactions [1,2], and interaction assays are crucial for the discovery, optimization, and analysis of various biotechnology application fields, including metabolic engineering [3–6], synthetic biology genetic circuits [7–10], the discovery of functional antibodies [11–13], enzymes [14,15], and drugs such as antibiotics [16–18].

Droplet screens are an ultra-high throughput technology that enables researchers to encapsulate interacting cells and reagents into thousands to tens of millions of microfluidic droplets with a pL to low-nL volume and efficiently analyze experimental results from each droplet, for example by fluorescence-based droplet sorting [19,20], DNA sequencing [11], cultivation [18], or imaging [21]. Droplet-based screens, in general, offer several advantages over more traditional methods, such as their ultra-high throughput, reduced reagent and plastic consumable use, cost-effectiveness, and minimized contamination risk [22], while improving the cultivability of microorganisms [22–24], spheroids, and organoids [1,25].

In the context of automated screening methods, droplets combine the advantages of the high-throughput and single-cell resolution of flow cytometry with the versatility of liquid-handling robots to use non-cell-bound reagents and dyes in assays [26]. Despite these advantages, the implementation of droplet screens also requires development time investment, and should ideally be chosen when more traditional screens are not well suited for the biotechnologically desired process. There are, however, many notable droplet-based biotechnology screens that harness the above advantages but are not necessarily based on interactions, such as different types of single-cell analysis [27,28] including quantification [29], transcriptomics [30], and genomics [31–33], to identify and characterize production strains, and directed evolution in droplets [14], which is often applied to enzyme and pathway optimization.

Interaction assays are a unique strength of droplets because they can usually not be performed with any other high-throughput method. The interactions in biotechnology are often based on the combinatorial testing of a limited number of molecules and cells, which can achieve interactions in the confined space of microfluidic droplets [2]. Many such interactions would not take effect quickly enough in well plates that contain microliters instead of picoliter volumes for the same number of molecules or cells to interact, and wells are also much more likely to contain contaminants in their larger volume. This volume-based advantage for interactions in

Figure 1



Schematic representation of the advantage of droplets to facilitate interactions between cells. **(A)** The compact volume of microfluidic droplets enables the rapid elevation of concentrations of molecules secreted by cells. Simultaneously, it promotes the swift encounter of a few encapsulated molecules with their target. This fosters cellular and molecular interactions to take place rapidly and intensively, such as the survival of a dependent auxotrophic cell, symbolized here by a yellow cell. **(B)** In contrast, larger assay volumes in combinatorial screens dilute the limited cell numbers available, leading to low concentration levels. This could hinder cells from effectively communicating or exchanging metabolites within a viable time frame. For instance, auxotrophic strains might not survive (depicted by gray cells) in the population due to this delayed interaction.

droplets is illustrated in Figure 1, based on a concrete example of metabolic interactions between auxotrophic bacterial strains by Tan et al. [2].

This review paper is a succinct exploration of the recent literature, primarily from the last two years, that highlights diverse applications and the significant advancements in droplet interaction screens. The aim is not to provide an exhaustive account, but this article type rather highlights recent examples and trends that showcase the technique's versatility and potential in both microbial and mammalian cell screens in biotechnology. We will also highlight recent methodology advances that expand droplet-based interaction screens to new environments, including cargo delivery inside the body, application of synthetic gene circuits in the natural environment, 3D printing, and more. It is the authors' belief that the interaction-focused perspective of droplet screens will lead to the discovery of previously unavailable screens that hold significant promise for scientific and commercial innovation.

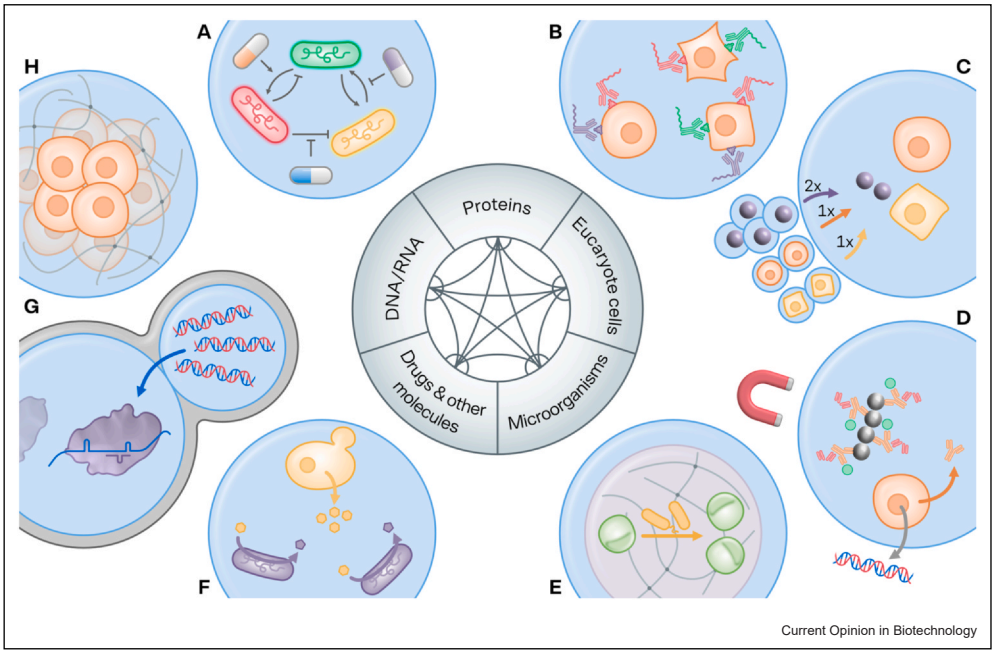
Recent highlights in droplet-based interaction assays

Droplet-based interaction assays have been successfully applied to study interactions between different cell types, proteins, DNA, RNA, drugs, and other molecules, as illustrated in Figure 2. The literature examples can be found for all 15 interaction categories shown in the figure's central graph, and some methods combine more than two types of interacting entities. While a wide spectrum of examples exists, most interaction screen types are still in the early stages of development, and we expect more research studies to be published in the coming years on this topic to mature biotechnological screening applications. Nevertheless, recent breakthroughs in droplet microfluidic screens for interaction assays have enabled new scales of bioanalysis and biotechnological product design. In the following paragraphs, we highlight the recent examples of different types of droplet-based interaction screens and discuss their potential uses to demonstrate the power and versatility of this emerging method trend and stimulate its application in research and industry.

Microfluidic droplets serve as a powerful tool for the quantitative exploration of complex interaction networks among cells and supplementary substances like drugs across various concentrations. The microbial network analysis shown in Figure 2A, for example, demonstrates an analysis involving three distinct, labeled auxotrophic bacterial cultures [34]. The potential for applications of such screens in modular genetic engineering approaches [40] is notable as it offers the possibility to widen and enrich the biochemical synthesis space through identifying productive co-cultures [41]. The scalable analysis of microbial metabolic interactions will further benefit the field of microbiome analysis and its biotechnology applications [42]. It is also worth mentioning promising methods for microfluidic co-culture, which are not performed in droplets [43].

The protein–protein interactions constitute another major area where a variety of droplet methods have demonstrated their value, a topic thoroughly reviewed in another work [44]. Figure 2B illustrates a method where

Figure 2



Schematic representation of different kinds of droplet-based interaction screens. The interactions of many biological entities can be screened in droplets, including proteins, such as antibodies and enzymes, eukaryotic cells, microorganisms, drugs, and other molecules, and DNA/RNA. At the center, binary combinatorial interaction possibilities are shown by connecting edges in a graph, and screens can incorporate more than two categories. Droplet schematics **(A-H)** highlight some examples of noteworthy interaction screens: **(A)** Quantitative network analysis of interactions between microorganisms and antibiotic drugs [34]; **(B)** Protein–protein interaction (co-occurrence) screens on the surface of different cell types [35]; **(C)** The active merging of droplets for interaction screens where all droplets have the same content type combination of cells and beads [36]; **(D)** Co-localization screen for functional antibodies that bind antigens to magnetic beads in droplets. The functional screen is followed by transcriptomics analysis in the barcoded droplets to sequence the functional antibody coding genes [11]; **(E)** Microbiota-algae co-cultivation screen in gel-microdroplets to select and sequence synergistic helper strains that aid the growth of algae culture [37]; **(F)** Yeast production cell-biosensor interaction in droplets to quantitatively indicate excreted product concentrations [38]; **(G)** DNA-protein interaction screen to detect all human viruses in multiple samples at once in a highly multiplexed diagnostic assay in a double–droplet trap array [39]; **(H)** Cell–cell interactions to form spheroids and organoids in gel microdroplets [25].

the co-occurrence of cell-surface proteins across different cell types is profiled utilizing a pool of labeled antibodies [35].

The encapsulation of interaction partners, like single cells or molecules, into droplets typically involves controlling the input concentration of each sample. This practice yields a stochastic encapsulation distribution in line with the Poisson distribution. As a result, achieving the preferred combination of different cells and molecules can be less efficient when dealing with larger quantities of interacting partners. To mitigate this challenge, several methods have been developed. One such strategy highlighted in Figure 2C involves actively selecting and merging encapsulated entities into the desired combinations [36]. This approach has been employed in a screen to detect cytokines on beads released during the interaction of two different cell types.

Interaction screens offer the potential to encompass an array of interaction types. Consider, for instance, the functional antibody screen depicted in Figure 2D. In this instance, droplets contain cell-expressing antibodies which, in turn, interact with an antigen and labeled antibodies, thereby co-locating fluorescence onto magnetic beads within the droplets. The screening process is continued by recovering functional cells and re-encapsulating them into droplets with reagents and DNA-barcoded gel microdroplets that facilitate the recovery of the transcribed genes of the heavy and light chain antibodies [11]. Beyond the bead-based assay, the authors also demonstrated a direct screen on target cells, akin to an earlier antibody gene-target cell screen conducted by a different research team [13].

Numerous types of interaction screens can be instrumental in enhancing the production of biotechnological products, be it by metabolic engineering [4,5] or directed evolution [14]. A prime example is the potential for increased yield in algal culture when cells are co-cultivated syntrophically with certain microorganisms as opposed to monocultures. Figure 2E features a screen that selects

synergistic bacterial strains through the utilization of the autofluorescence of proliferating *Chlorella sp.* colonies in gel microdroplets [37]. The study also indicated that co-cultures in droplets could be incubated for a duration of up to 60 days. An analogous co-culture strategy was employed to culture microorganisms in the laboratory that did not grow in conventional cultures [45].

Where autofluorescence and fluorescent labels are not readily available, synthetic biology biosensors can be incorporated into droplet screens to quantify the productivity of target cells by interaction [9] for metabolic engineering applications. The method outlined in Figure 2F leverages droplets for yeast metabolic engineering to quantify the product (p-coumaric acid) excretion into the droplet with biosensor reporter cells. These biosensor cells harbor an operon with a transcription factor that responds to the product and encodes for a fluorescent protein signal [38]. In more recent developments, a cellular biosensor was designed to screen for the secretion of the industrial chemical (3-Hydroxypropionic acid) in droplets [46], and another recent biosensor platform evolved the yield (of erythromycin) from genetically difficult to engineer actinomycetes production strains [47].

Droplets do not always need to be sorted in order to be characterized. As demonstrated by the diagnostic assay illustrated in Figure 2G, it is possible to screen multiple clinical samples for all 169 human-associated viruses (i.e. viruses with > 10 published sequences) in a protein-DNA interaction screen. Initially, all DNA-amplified patients, sample-derived droplets and the Cas13-based virus detection mix droplets were color barcoded. Subsequently, droplets were trapped pairwise side-by-side in a large double-droplet trap array. Finally, the color barcode combinations were imaged before and after the droplet merger, measuring the color barcodes and a fluorescent readout where the target viral sequences were present [39].

Another notable application of the DNA-protein interaction screen type in biotechnology deserves mentioning. This involves the interplay between genetic fragments and the enzymes and metabolites of cell-free extracts, which serves to express and evaluate synthetic biology constructs within droplets. This method can significantly enhance the efficiency of engineering test cycles [8,10].

Mammalian cell–cell interactions are crucial for the formation of physiologically relevant tissue, a critical area for the enhancement of early-stage clinical trials. The droplets are suitable for the generation of both spheroids and organoids, frequently in gel microdroplets, such as Matrigel, agarose, alginate, and gelatin, among others (refer to Figure 2H), or within gel-shell capsules [25].

Expanding the applications of droplet-based interaction screens

As we have seen, high-throughput interaction screens based on microfluidic droplets can be performed with a versatile range of droplet components, such as cells and molecules, but also magnetic beads, color or DNA barcodes, and gels. Notably, advancements in gel microdroplet materials have paved the way for a broader application of droplets in cell cultures, multi-step procedures, and streamlined molecular processing protocols. In this section, we highlight several innovative material developments that promise to broaden the reach of droplet-based interaction screens, catering to a wider audience and novel environments, including interactions occurring between droplets and their surrounding milieu.

For example, coated microgels (alginate hydrogels with an alginate and polyacrylamide coating) have been used to physically contain genetically modified microorganisms [48], allowing them to be incorporated into the natural environment in a controlled manner, as illustrated in Figure 3A. Even after 72 hours, no microorganism escape was detected, but nutrients and signal molecules were successfully exchanged between the environment and adjacent capsules. Such capsules provide the basis for syntrophic interaction screens with the environment.

A widely used advantage of gel-microdroplets and double emulsion droplets is that they can be processed in an aqueous environment, and therefore sorted in a regular flow cytometer [49,54]. Figure 3B illustrates the use of double emulsion for cell sorting in a commercial flow cytometer. Such machines are available in a wider range of research laboratories and can therefore increase access to droplet-based interaction screens. Unfortunately, the generation of double emulsions and gel microdroplets is more challenging than that of regular droplets.

Cells can be enclosed in shell droplets, which can be 3D-printed and cross-linked into cell-containing structures [50], as shown in Figure 3C. This process opens new possibilities to place interaction partners in space for interactions between different encapsulated organisms and their environments. This technology may also enable the co-design of organs on a chip with a spatially and compositionally engineered microbiome.

A key environment for the placement and release of drugs and organisms is the human body. Indeed, droplets can be used for injection and targeted delivery [55], such as injecting gel microdroplets loaded with hair formation cells and plasma with platelets into the hairless tissue for regeneration [51]. The method illustrated in Figure 3D utilizes specialized hydrogels (gelatin

Figure 3

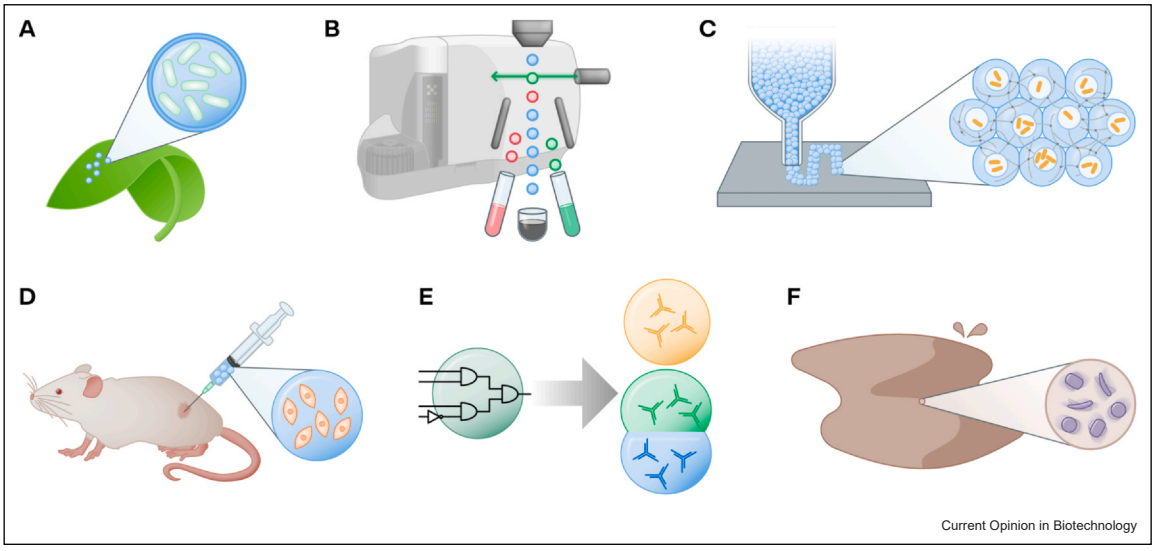


Illustration of emerging droplet-based interaction screens facilitated by using new materials. **(A)** The biocontainment of microorganisms is enabled by an alginate-polyacrylamide coating that allows the controlled incorporation of these organisms into the environment [48]. **(B)** Cell sorting using a flow cytometer and double emulsion [49]. **(C)** The bioprinting of cells encapsulated in shell droplets delivers more precise spatial cell-cell interactions [50]. **(D)** Hair follicles delivered by microfluidic droplets of GelMA and chitosan hydrogel present a new method of targeted delivery [51]. **(E)** DNA droplets contain a genetic circuit capable of sensing the presence of microRNAs by disrupting the homogeneous distribution of DNA, separating them into three distinct droplets [52]. **(F)** Wastewater treatment using micromotors whose generation was assisted by microfluidic droplets and which catalyze the degradation of organic waste [53].

methacryloyl [GelMA] and chitosan) for targeted delivery. The delivery systems can be designed in several layers and with multiple functions, such as featuring a self-renewable hydration layer (based on liposomes) for delivery into joints [56].

Finally, the droplets can be designed to actively respond to or actuate their environment. While these examples still tend to be in the proof-of-concept stage, they allow us to think about new types of interactive screens. Figure 3E shows the example of a responsive droplet used for a bio-based assay, a single droplet made of a purpose-designed DNA gel with different DNA motifs and linkers to bind them. The linkers feature a base complementarity for four different target miRNAs. In the presence of target miRNAs, the linkers hybridize with them, which unbinds the motif and compartmentalizes the droplet into three single-motive droplets [52]. Another example of a responsive microfluidic droplet is the stimulated drug-content ejection of a multiphase Janus microparticle [57]. The droplet-based micromotors are not responsive but are constantly actuated [58], which can include the catalytic interaction properties that are used for organic waste degradation in wastewater treatment [53] (Figure 3F).

Conclusion

Droplet microfluidics has firmly established itself as an unrivaled high-throughput approach for screening in the realm of biotechnological applications. The significance of droplet-based screens is surging in parallel with the growing arsenal of available methods and tools. A noteworthy trend emerging in this field is interaction assays. Given their small volume, droplets are perfectly suited for facilitating and quantifying interactions, as demonstrated by numerous high-impact studies recently. We've brought attention to various types of interaction methods, spanning protein–protein, cell–cell, DNA-protein, and drug-microorganism interactions, amongst a host of other studies blossoming in this rapidly expanding area.

Beyond droplet contents, the material composition, processing protocols, and novel strategies for introducing droplets into the environment are being explored, such as deploying them in more widely available instruments, within the human body for organism and drug delivery, or even in natural settings. These innovative methods unlock new avenues for interaction studies where the droplet content or the responsive droplet material itself can interact with the biological milieu. We anticipate that this will catalyze the emergence of new biotechnological applications.

Despite the many benefits of droplet microfluidic screens, several challenges remain, including the need for experimental knowledge, specialized equipment, and interdisciplinary communication skills [59]. The

advanced droplet selection setups are particularly promising but not widely available or easily replicated [60]. Additionally, methods often need to be re-optimized and benchmarked, which requires time investment. It is therefore particularly practical to focus method development efforts on screens that are unique for droplets such as interaction assays, which do not simply compete with other methods over a cost or efficiency margin.

As a final note, it is important to recognize the growing significance of alternative measurement methodologies in single-cell analysis, such as mass spectrometry, Raman spectroscopy, and impedance spectroscopy, among others. Our review does not cover these methods, because these techniques have yet to make their major impact within droplet-based interaction studies, nevertheless, their potential to enhance this field is clear by offering direct label-free metabolomics, proteomics and the electrochemical analysis of droplet contents. We anticipate that these complementary tools will increasingly intersect with droplet-based systems in the future, broadening our scope of high-throughput screening capabilities.

As we move forward, the potential of droplets for interaction screens continues to grow. This brief review hopes to stimulate further research and industrial use of these promising techniques, and we look forward to the discoveries and innovations that will undoubtedly continue to emerge from this exciting field.

CRediT authorship contribution statement

Carolus Vitalis: Conceptualization, Data curation, Visualization, Writing – original draft. **Tobias Wenzel**: Conceptualization, Data curation, Visualization, Writing – original draft, Funding acquisition, Supervision, Writing – review & editing.

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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- •• of outstanding interest

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