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VIROLOGY (A NICOLA, SECTION EDITOR)

Microfluidics: an Untapped Resource in Viral Diagnostics and Viral Cell Biology

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Abstract

Purpose of Review Microfluidic platforms have become valuable tools in a wide variety of research environments. With the ability to allow detailed examination of an array of cell biological processes, their use in the field of virology is becoming progressively more common. This review will discuss the potential applications of microfluidics in viral cell biology and explore the potential of these techniques to alter the way in which we study the biology of infection.

Recent Findings In recent years, scientists have utilised microfluidic platforms for detailed study of the viral life cycle. Microfluidic technologies have allowed investigation of viral infectivity, measurement of fusion kinetics, and monitoring of viral responses to neutralising compounds. In addition, microfluidic platforms represent promising new clinical tools with applications in diagnostics and drug screening.

Summary Although the potential of microfluidics in virology is beginning to be realised, it has certainly not been fully explored. While not a replacement for macroscale investigative techniques, microfluidic platforms have the potential to be utilised alongside systems biology to provide novel methods of detailed virus study, with unique advantages.

Keywords Virus microfluidics · Virus entry · Viral cell biology · Fluorescence imaging · Single particle · Single-cell analysis

Introduction

Microfluidics can be thought of as both a science and a technology. The term specifically describes a range of techniques concerned with the precise control and manipulation of fluids, within microscopic channels [1]. This relatively straightforward concept underpins an array of biological research techniques, from flow cytometric and DNA analyses to enzyme and immunoassays [2, 3]. With the potential to expand experimental approaches in a wide variety of scientific fields, microfluidics could represent a novel set of explorative techniques in the fields of cell and infection biology.

In order to fully appreciate the potential of microfluidic platforms in scientific research, it is first necessary to understand the basic principles and components of a typical microfluidic device. Microfluidic technologies are primarily based upon the theories of fluid mechanics: While fluid flow on a macroscale is likely to be turbulent and unpredictable, fluids on a microscale move consistently in laminar flow [3]. Solutions in laminar flow make attractive entities for experimentation since they are highly predictable and therefore offer researchers the opportunity to precisely manipulate experimental environments (Fig. 1(A)).

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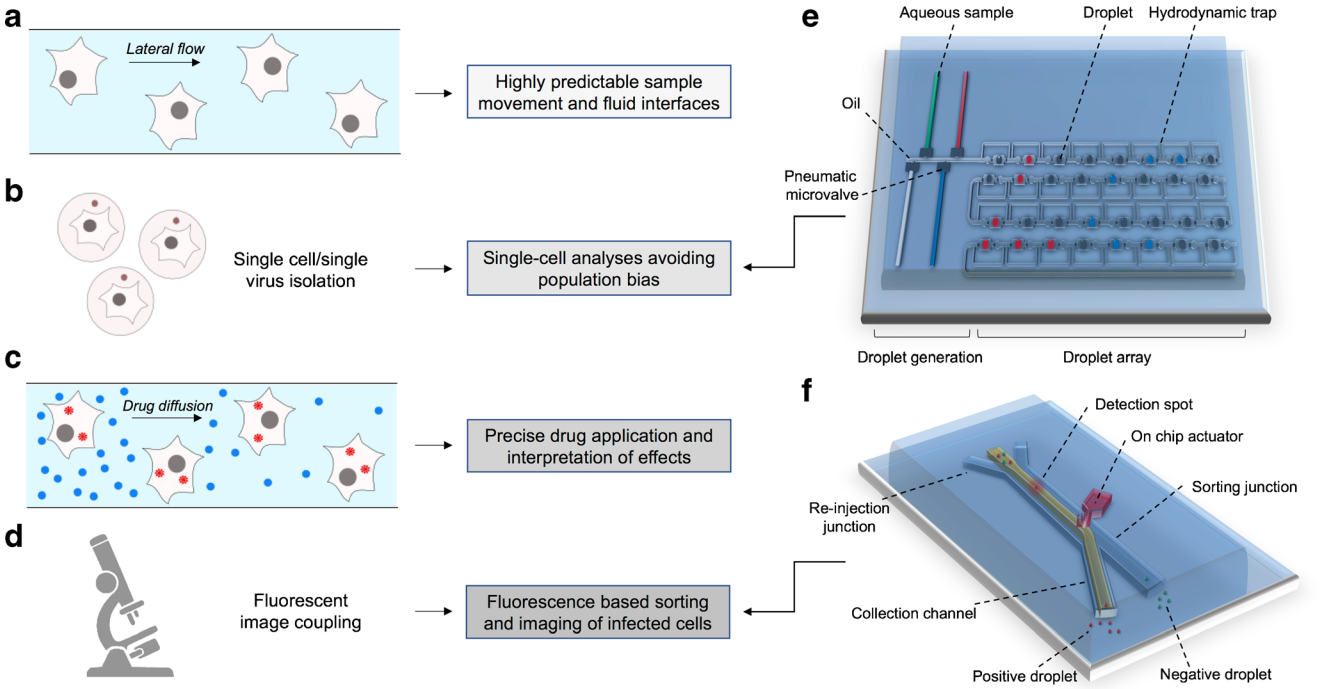

Fig. 1 (A–D) Typical features of microfluidic set-ups and their advantages over traditionally used techniques. (A) Fluids within microchannels move uniformly in laminar flow, creating highly predictable experimental environments. (B) Droplet-based microfluidic systems allow isolation of single cells and viruses within specialised microenvironments. (C) Precise application and subsequent diffusion of test compounds allow for the creation of precise concentration gradients. (D) Coupling microfluidic devices to fluorescent microscopes can allow both live imaging of dynamic infection processes and sorting of cells based on infection status. (E) A typical droplet-based microfluidic chip with key components labelled. (F) A fluorescence-based, microfluidic cell sorting device with key components labelled

Microfluidic devices typically consist of mechanical pumps that facilitate fluid motion and a network of microchannels, within which target fluids are transported and experimental interfaces are present [4] (Fig. 1(E, F)). Most systems also contain devices that regulate physical conditions within the platform such as temperature and pH level. Fluids within a device typically move either in continuous, laminar flow, or as finite volumes suspended in sheath fluid, which is known as droplet-based or digital microfluidics. Droplet-based microfluidics, a specialised platform often utilised for single-cell analysis, has become a key research technique in cell biology (Fig. 1(E, F)). These platforms create homogeneous, microscopic droplets within an immiscible sheath fluid, which serve as specialised microenvironments for experimentation. Reprogrammable and reusable static droplet array (SDA) microfluidic devices have been recently reported, with the ability to allow precise droplet formation, transportation, fusion, and observation [5].

Polydimethylsiloxane (PDMS) is generally the material of choice for microfluidic chip fabrication due to its compatibility with soft lithography techniques for microstructure moulding. PDMS is both gas permeable and transparent, making it ideal for both cell culture and live imaging [6]. The physical characteristics of PDMS, including its high elasticity, are also particularly advantageous for cell trapping and alignment within microfluidic devices, to be used for imaging studies [7].

Microfluidic platforms can provide numerous advantages to biomedical scientists, over traditionally used experimental techniques (Fig. 1(A–D)), especially when sample volumes are a limiting factor. By drastically reducing the scale on which experiments can be performed, they decrease both sample and reagent consumption, cutting costs and allowing analyses, even when a limited volume of sample is available [8]. In numerous biochemical assays, limited sample size can result in unreliable results. Sample analysis using a microfluidic set-up may circumvent these issues in that samples are minimally diluted and detection thresholds are significantly lowered due to the reduced reaction volumes involved [2]. The precise regulation of experimental conditions provided by a microfluidic platform also provides researchers with a higher degree of control over the physical and chemical environment during their investigations. In the field of viral cell biology, where both temperature and pH level can greatly impact infection dynamics, these characteristics are particularly valuable. In addition, as a consequence of laminar flow characteristics, a high degree of spatial and temporal resolution can be achieved using microfluidic technologies, via precisely regulated diffusion of samples or test compounds [4] (Fig. 1(C)). For example, precise chemical concentration gradients created in fluid streams adjacent to those in laminar flow provide a stable and accurate method for drug delivery within a microfluidic chip [9, 10].

Coupling microfluidic devices to live imaging platforms has also proven to be highly useful, allowing the extraction of both qualitative and quantitative data, during a single experiment [2] (Fig. 1(D)). Microfluidic set-ups also allow scientists to perform thousands of individual experiments in parallel within a single device, increasing the throughput of numerous investigations [4]. Since microfluidic platforms allow for the collection of complex dynamic information during imaging experiments at high spatial and temporal resolution, they may in some instances provide significant advantages over macroscale experimental approaches. While collaboration may be necessary to set up and establish an efficient platform, in studies where minimal sample use and environmental control are paramount, application of microfluidics may provide a unique investigative platform with enhanced capabilities.

With a broad range of applications from 3D cell culture and single-cell analysis to protein crystallisation [11] and drug screening, microfluidic technologies have an enormous range of uses that have collectively benefitted an array of industries in previous years. In recent times, microfluidic techniques have been successfully implemented in the field of cell biology for the study of the cellular transcriptome and proteome and examination of the mechanical properties of the cytoskeleton, all at single-cell resolutions [2]. While several microfluidics-based set-ups have become mainstays in the field of biomedical science, tailored application of microfluidic techniques to the study of viral biology is a somewhat neglected field of research. In recent years, several studies have implemented microfluidic platforms for the study of viral cell biology, with promising results. Further application of microfluidics in the field and development of novel experimental platforms for use alongside systems biology-based approaches has the potential to alter the landscape of investigative techniques in the field of infection biology research.

Microfluidics in Virological Research

Microfluidic Platforms in Viral Diagnostics

The development of point-of-care viral diagnostic devices remains an active area of clinical research and represents an essential precursor to successful treatment for a wide variety of human pathogens. Notably, several recent reports describe the use of CRISPR-based nucleic acid amplification methods for rapid diagnosis of HPV, dengue, and zika viruses [12–14]. Similarly, in recent years, microfluidic technologies have been investigated for clinical diagnostic purposes, with several platforms demonstrating efficacy for the correct and swift identification of viral infections.

One recent study demonstrated that parallel detection of multiple viral pathogens can be achieved using a microfluidic chamber containing numerous channels within which, viral DNA may be amplified to form hydrogels. Following hydrogel formation, blockages in fluid flow and subsequent viral infection can be detected via dye injection [15]. The fact that RNA virus detection relies on previous reverse transcription to produce detectable cDNAs may be a limitation of this technique. However, since DNA constructs are likely to be stable for some time within a microfluidic chip, this system may be compatible with point-of-care diagnoses should chips be prepared and available in advance. Moreover, since the described methodology could be applied for the detection of a wide variety of viruses, this relatively simple set-up may hold potential for viral diagnostics in regions where sophisticated experimental machinery is unavailable and/or there is a lack of electricity. Validation of this detection method, using sera from virus-infected patients will be the next important step to determine its clinical relevance.

Several microfluidic set-ups utilising antibody-based viral detection have also been recently described [16, 17•]. In one of these studies, a microfluidic platform for the diagnosis of dengue virus (DV) via host antibody recognition was shown to improve detection thresholds relative to previously used diagnostic techniques, including PCR and enzyme-coupled immunoassays [16]. However, since adaptive antibody responses in an infected host can take several days to develop post-pathogen exposure, host-generated antibodies may be less reliable infection markers for patients presenting with clinical symptoms immediately after infection. This study, however, demonstrated an important proof of principle, in that microfluidic technologies enabled sensitive detection of target antibodies. In a similar experimental platform, monoclonal antibodies directed against viral nucleoprotein (NP) were utilised within an integrated microfluidic chip for the identification of influenza A or B virus (IAV/IBV). Samples containing influenza viral particles were first bound to magnetic beads and subsequently incubated with fluorescently tagged, anti-viral antibodies. Samples could be imaged via fluorescence microscopy to allow swift detection of infection following viral immunofluorescent labelling [17•]. One alternative to antibody detection, which can also be utilised by microfluidic platforms, involves aptamers, peptide molecules that can be designed to target any given protein [18, 19]. A recent study investigating the use of an integrated microfluidic system for diagnostic purposes utilised aptamers against IAV (H1N1) for viral detection [20•].

Collectively, these studies demonstrate that microfluidic techniques allow for the incorporation of a variety of viral detection methods. While the optimal microfluidic platform for clinical diagnostic purposes remains to be ascertained, microfluidic platforms themselves represent novel technologies with several strong selling points for clinicians.

Firstly, the pathogen detection time in numerous diagnostic tests utilising microfluidics tends to be extremely short, with

infections confirmed within 15–30 min [15, 16, 20•]. Since prompt treatment following infection is often paramount for the success of anti-viral agents, point-of-care devices which do not require lengthy sample processing procedures may greatly improve the success of treatment. In addition, detection thresholds in microfluidic-based systems have been demonstrated to be significantly lower than those in conventional assays for viral diagnosis such as enzyme-based immunoassays and PCR techniques for molecular diagnosis [15, 16, 20•]. The ability to correctly diagnose even low-level infections gives microfluidic platforms a huge potential in the field and may significantly reduce false negative diagnostic tests. These factors make microfluidic devices ideal candidates for the development of novel, clinical diagnostic tools.

In addition to detection, viral concentration in clinical samples is possible using microfluidic techniques [21]. One such system utilised microfluidic channels which contained both fluid and gaseous compartments, separated by a hydrophobic membrane. As sample fluid moves through the channel, evaporation through the pores in the membrane facilitates sample volume reduction and pathogen concentration [21]. Since low concentrations of pathogenic material are a common problem in diagnostics, pre-processing with such a microfluidic device may increase the sensitivity of pathogen detection.

Microfluidics in Anti-viral Drug Discovery and Vaccine Development

Microfluidic technologies are a promising set of techniques for drug discovery and have been employed in several fields of research to test compound efficacy. Significant validation of the usefulness of microfluidics in drug screening comes from the field of cancer research, in which several studies have utilised microfluidic chips for high-throughput screening (HTS) of numerous anti-cancer agents [22, 23]. In the field of virology, microfluidic techniques have yet to be widely applied to screening experiments; however, the proof-of-principle studies in cancer research indicate that these platforms could be valuable research tools. By providing an experimental system with increased biological relevance and reducing the overall sample volumes required for experimentation, microfluidic platforms have the potential to surpass a variety of currently used screening techniques in the field of anti-viral drug discovery [24]. Precise manipulation of test conditions coupled with single-cell analyses also makes microfluidic platforms unique in their ability to precisely control the microenvironment, a factor that greatly influences experimental outcomes during cell biological investigations of drug efficacy.

Notably, two recent studies into the infectivity of murine norovirus (MNV) have utilised droplet-based microfluidic platforms to test the efficacy of neutralising antibodies and investigate their impact on virus-cell interactions [25, 26]. In comparison to current techniques used to measure viral load, including plaque assays and quantitative polymerase chain reaction (qPCR) analyses, microfluidic technologies significantly reduce reaction volumes while providing an increased amount of information, by demonstrating the heterogeneous responses to inhibitory compounds within a cell/viral population. By allowing parallel identification of both sensitive and resistant viruses, these screening systems also have the potential to estimate the potential for viral resistance to novel therapeutic agents prior to their clinical use.

The field of vaccine development may also benefit from microfluidic technologies as demonstrated by a recent study which used a droplet-based platform to perform parallel screening and sorting of human immunodeficiency virus 1 (HIV-1) virions by epitope expression [27••]. This system essentially acts as a miniaturised flow cytometer, enabling fluorescence-based sorting of viral particles possessing antigenic envelope proteins. Genomic sequencing of envelope proteins in viral populations expressing antibody-binding epitopes has the potential to reveal novel candidate targets for HIV vaccines [27••].

While flow cytometric analyses of viral particles have been previously difficult due to size restrictions, microfluidic devices allow miniaturisation of this technology and its subsequent application to smaller viruses outside the threshold for detection via traditional FACS methods. With the potential to allow high-throughput epitope screening using vast libraries of viruses, these techniques may reform the field of vaccine development, for viruses as well as for other microorganisms.

Microfluidic Platforms for the Study of Viral Cell Biology and Virus-Host Interactions

In recent years, several virologists and cell biologists alike have utilised microfluidic technologies for the study of viruses and their interactions with host cells. Moreover, it is becoming apparent that microfluidic platforms offer unique experimental set-ups for a broad range of biological investigations, which cannot be mimicked by widely used macroscale techniques.

Perhaps the most widespread application of microfluidics in virology lies in the study of viral entry processes, which can be performed within microfluidic devices with a high degree of precision.

Microfluidic devices have been utilised in a collection of research studies, to investigate influenza virus fusion kinetics [28–30, 31•]. In one such study, microfluidic flow cells containing synthetic vesicles with the ability to bind viruses in the absence of native receptors were utilised to investigate the dependence of viral fusion on receptor binding [28]. Lipid mixing measurements were used to record fusion events in this system, which confirmed that receptor binding and fusion are in fact separate dynamic processes in viral entry [28]. A

second investigation explored whether differential expression of influenza virus haemagglutinin (HA)/neuraminidase (NA) proteins in synthetic vesicular stomatitis virus (VSV) particles could predict efficient viral fusion [30]. Researchers found that the specific pattern of HA/NA expression in an individual virus could reliably predict fusion kinetics in target membranes. A further study examined the links between membrane composition and viral fusion kinetics [31•]. This research revealed that IAV fusion is significantly dependent on membrane cholesterol content as well as sialic acid donor availability [31•]. Since viral fusion behaviours are highly dependent on pH level, the precise manipulation of microenvironmental pH in both space and time offered by microfluidic devices would have been a unique advantage for each of these studies.

These kinetic fusion studies could also be applied to drug screening experiments, as suggested by Mashagi et al., in a recent paper demonstrating that a droplet-based microfluidic platform could efficiently measure IAV fusion kinetics with a high degree of temporal resolution [29]. Droplet-based systems that provide dynamic information regarding the phase of viral replication affected by inhibitory compounds have the potential to provide researchers with a large amount of information regarding the mechanisms of action of candidate anti-viral compounds and could serve as valuable tools for HTS in the development of anti-viral therapeutics.

Microfluidic technologies have also been applied to investigate viral replication. Several studies have used microfluidic chips, coupled with fluorescent imaging to quantify viral replication in a variety of cell types [32, 33]. That microfluidic techniques have proved highly compatible with live imaging experiments further validates their usefulness for this purpose.

Microfluidics in Single-Cell Analyses

As well as population-wide information, droplet-based microfluidic platforms can be used for single-cell analysis of infective processes. One recent study utilised a microfluidic chip connected to a fluorescence microscope to image poliovirus infections in real time, in thousands of individual cells [34]. The study revealed that microfluidic techniques could allow efficient visualisation of thousands of individual infection events and subsequent examination of variable infection dynamics within single cells originating from the same population. This study also utilised their single-cell platform for drug screening experiments, demonstrating that population-wide effects of candidate inhibitory compounds do not accurately portray the between-cell variation that exists within a heterogeneous population of cells and viruses [34]. With the ability to meticulously regulate cellular microenvironment and compound application, this platform provides an efficient technique for anti-viral drug screening in comparison to macroscale studies, where environmental factors and drug diffusion are likely to be less controlled.

In addition to acting as a stand-alone research platform for single-cell analyses, microfluidic set-ups can also serve as valuable tools for cell isolation, prior to detailed analyses. A recent study utilised a droplet-based microfluidic platform which allowed barcoding of cells based on RNA expression and subsequent detailed analyses of cellular transcriptomics [35••]. An array of studies have also performed proteomic and genomic characterisation of single, virus-infected cells [36]. In each of these studies, cell isolation was a fundamental prerequisite to single-cell analysis. Microfluidics may provide an efficient technique for sequestering single cells, prior to detailed investigations of genetic and proteomic phenotypes using a wide variety of techniques. This was demonstrated by a recent study in which the cellular transcriptome in cells susceptible to HIV-1 infection utilised microfluidic chips for single-cell isolation [37].

The wide variety of cell biological processes in infection which could be investigated using microfluidic set-ups such as those discussed above provides an insight into the enormous potential of these techniques in virus research. Using microfluidic chips to study infection dynamics may provide mechanistic information regarding underlying biological processes mediating the effects of inhibitory compounds or manipulation of host-cell factors, with a higher degree of spatial and temporal resolution than traditional approaches. In addition, single-cell analyses are able to circumvent population bias and provide detailed snapshots into the dynamic infection process in unique cell and viral populations, contained within specialised microenvironments.

The Advantages of Microfluidic Platforms in Viral Cell Biology

While microfluidic technologies provide useful platforms for experimentation in the field of virology, their widespread application depends on their ability to offer significant advantages over widely available and well tested techniques. The collection of studies recently performed in the field points to valuable uses for microfluidic platforms in particular areas of cell biological and virological research.

While studying viral infections at a population-wide level has provided valuable information regarding infection dynamics and facilitated investigation into the effects of countless anti-viral agents, these macroscale studies will always be associated with some degree of population bias. Overlooking important phenotypical differences in infection dynamics within a cell or viral population means that macroscale techniques often miss unique characteristics which may convey altered susceptibilities to infection or drug treatment. These rare characteristics themselves have the potential to reveal

important information regarding the mechanisms which underlie host cell permissiveness to infection, viral resistance to therapy, and viral evolution. Studying single-cell infections using a droplet-based microfluidics system in combination with systems biology investigations could therefore provide a level of detail previously inaccessible to macroscale techniques alone. Microfluidic platforms may also prove uniquely advantageous for live, viral imaging experiments for inhibitory compound screening. Visualising infection dynamics in single, drug treated cells could provide a wealth of information in HTS experiments regarding both compound efficacy and mechanisms of action.

In contrast to macroscale techniques, microfluidic single-cell analyses offer superior control over numerous experimental conditions. Precise concentration gradients of candidate inhibitory compounds as well as viral particles can be created with ease within a microfluidic chip and physical factors which may alter infection outcome such as temperature and pH that can also be precisely manipulated. In macroscale studies, these conditions are often difficult to regulate precisely, and some variation in microenvironment will always exist between individual cells within a population. The ability to precisely control a given experimental environment is therefore an extremely useful and unique aspect of microfluidic-based platforms, which in combination with macroscale studies may allow development of a more controlled and biologically relevant model system for studying infection dynamics.

Conclusions

While advancing research techniques in a wide variety of scientific fields, microfluidic platforms remain an untapped resource in the field of virology. With the potential to improve investigatory techniques in viral diagnostics, drug discovery, and vaccine development, microfluidic platforms may represent a novel clinical toolset with significant impact in the field. Viral cell biology research may also benefit significantly from wider application of microfluidic techniques. With the ability to provide detailed insights into the unique and dynamic interactions between single cells and viruses, these platforms could serve to enrich our understanding of the cell biology of infection. By allowing precise and highly regulated spatial and temporal application of both viruses and drugs, microfluidic systems also offer more precise control and manipulation of several key experimental parameters. The high degree of control over cellular microenvironment offered by droplet-based microfluidic systems may be unparalleled by macroscale investigations. Since viral infection is a complex and highly dynamic process, significantly affected by the physical and chemical environment, studies into infection biology should ideally take place within a stable and controlled experimental compartment, such as that provided by a microfluidics system. For these reasons, microfluidic platforms provide novel tools for studies in infection biology, with unique capabilities.

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Compliance With Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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