Single cell virology using drop-based microfluidics

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1. Abstract

Viral infection studies traditionally rely on population-level approaches in which cells are cultured and infected in well plates. However, these methods often overlook the heterogeneous dynamics of infection. Single-cell virology methods have advanced our understanding of cellular physiology and viral infection, enabling the observation of underrepresented phenotypes. Here, we present methods for investigating single-cell viral infections using drop-based microfluidics. This allows for single-cell assays to be conducted in emulsions of monodisperse, picoliter-sized aqueous drops in oil. Specifically, we detail the use of drop-based microfluidics to investigate the dynamics of herpes simplex virus (HSV-1) infection in single neurons. We use microscale hydrogel beads to culture individual murine SCG neurons, which are subsequently encapsulated in drops containing a dual fluorescent-reporter HSV-1, enabling real-time observation of viral gene expression and replication. Our results indicate that increasing the inoculating dose leads to an earlier onset of viral gene expression and more frequent productive viral replication. Additionally, we describe the application of drop-based microfluidics to quantify the viral burst size, or the number of viral particles released from an infected cell, for both H3N2 and H1N1 strains of influenza A virus (IAV). We introduce a microfluidic method called droplet quantitative PCR (dqPCR) for the rapid measurement of influenza virus numbers produced by thousands of individual cells. Our findings reveal that only a small proportion of infected cells are responsible for producing a significant portion of the total viral population. By incorporating drop-based methodologies into future studies, we can gain a deeper understanding of the role of diversity in rapidly evolving viruses.