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CAPDROP: A NOVEL METHOD FOR PERIPHERAL BLOOD scRNA-seq

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Droplet microfluidics is a high-throughput technology for analyzing individual cells isolated in nanoliter-volume droplets. This approach addresses cell heterogeneity challenges, enabling the comparison of cell states and types in complex samples [1]. However, conventional droplet-based systems encounter limitations when it comes to executing multi-step operations which can be challenging to implement.

Our research group developed a microfluidics-based technique for single-cell isolation in semi-permeable capsules [2,3], that selectively retains large molecules, such as genomic material or mRNA, while allowing small molecules, such as enzymes or other reaction components, to passively diffuse throughout the shell. As a result of this selective permeability, it becomes possible to perform multi-step reactions on millions of individual cells. When compared to other systems with similar properties, the semi-permeable capsules exhibit improved retention of encapsulated cells and yield higher quantities of whole-genome amplification [2].

Here, we adapted capsules to create a novel single-cell RNA sequencing (scRNA-seq) platform to profile fragile and hard-to-capture cells. Traditional droplet-based scRNA-seq faces challenges in sequencing cells that are sensitive to environmental factors. One of these cell types is neutrophils which are characterized by a high concentration of internal RNases, which, upon stimulation, can degrade the transcriptome of both the neutrophils and the surrounding cells. Taking advantage of capsules, we demonstrated that it is possible to effectively neutralize RNases effect and subsequently recover diverse cell types. The capsules carrying purified cellular RNA are used as microreactors for barcoding and subsequent library preparation. We term this new technique C̈apDropänd show that CapDrop recovers the peripheral blood cell composition close to theoretical values, therefore addressing a significant limitation of droplet-based scRNA-seq technology. Our findings underscore the potential of the CapDrop platform as a promising method for sequencing sensitive biological samples. The flexibility and efficiency offered by CapDrop open new avenues for exploring cellular heterogeneity and advancing single-cell RNA sequencing methodologies.

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