A tale of two platforms: HIVE scRNAseq solution recovers complete biology in comparison to droplet method.

Key takeaways

- HIVE™ scRNAseq Solution enables complete recovery of fragile cell populations, as opposed to droplet-based platform
- Nearly 100% reduction in granulocyte recovery when using droplet platform

Introduction

Many single-cell RNA sequencing (scRNAseq) approaches exist; most notable are droplet and picowell-based technologies. Droplet-based, microfluidic platforms isolate a single cell and barcoded transcript-capture bead within an oil droplet. In picowell-based platforms, both cell and bead are isolated within a well. As part of the HIVE™ scRNAseq Solution, the HIVE device, in which sample capture occurs, contains thousands of picowells pre-loaded with capture beads. Single-cell suspensions are gently loaded into the HIVE device, either by gravity or low speed centrifugation [Figure 1]. In droplet-based systems, cells are subject to shear forces[1] from the microfluidic device, which may stress cells and result in poor recovery of fragile cell types such as granulocytes. Additional logistical challenges exist with droplet-based technologies, including higher costs due to a need for specialized equipment, difficulty collecting and processing samples in different locations, restrictions with sample loading volumes, an inability to transport equipment across biosafety levels, clogging of microfluidic channels, and limitations with customization of reagents like lysis buffers.

For research use only. Not for use in diagnostic procedures.

Here, we present data generated by loading the same sample – fresh human blood from a healthy donor – into the HIVE device and a commercially available droplet-based platform. The results show superior recovery of fragile granulocytes when using the HIVE™ scRNAseq Solution.

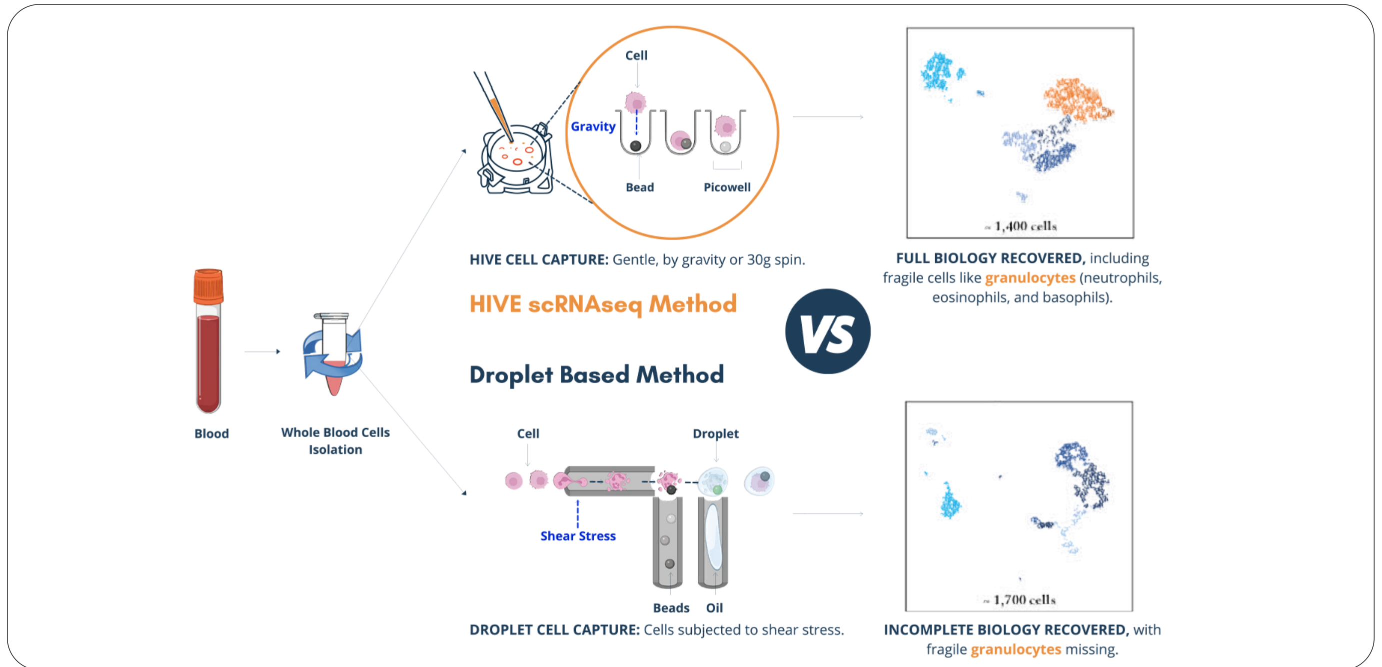


Figure 1. Overview of HIVE scRNAseq vs. Droplet Methods

Methods

Fresh human blood from a healthy donor was used in this study. Red blood cells (RBC) were depleted from 1 mL of fresh blood by size selection using a sterile Acrodisc White Blood Cell Syringe Filter (Pall Laboratory, #AP-4951). With this filter, all leukocyte populations were isolated, including granulocytes, which are lost with other RBC depletion methods such as Ficoll treatment.

Single-cell libraries from fresh samples were immediately generated following the HIVE™ scRNAseq Solution Sample Capture and Processing protocols [outlined in Figure 2].

Count matrix files were generated using BeeNet™ software, and data were analyzed using Seurat v4.0.5.

Single-cell libraries were also immediately generated after fresh cells were loaded into the droplet platform and processed according to vendor provided protocols. Count matrix files were generated using the vendor provided primary analysis pipeline, and data were analyzed using Seurat v4.0.5. Honeycomb Biotechnologies' filtered blood dataset was used to automatically annotate both HIVE and droplet cell-types.

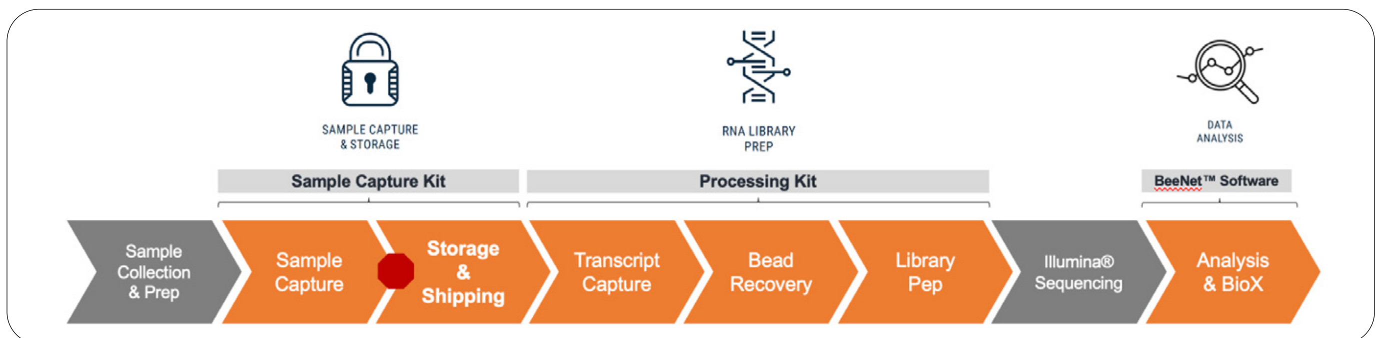


Figure 2. HIVE™ scRNAseq workflow

Results

After standard thresholding for high-quality cells (> 400 genes and > 800 transcripts) for each dataset, UMAP plots of single cells from both platforms were generated and colored by cell type [Figure 3]. Data from comparable numbers of cells recovered from both methods were considered. Sixteen cell-types were identified. All granulocyte populations (neutrophils, eosinophils, and basophils) were recovered by the HIVE device; however, those cell-types are missing from the droplet data [Figure 3]. This observation is corroborated by granulocyte feature plots, in which the robust aggregate expression of granulocyte markers is seen for HIVE data but not for droplet data [Figure 4].

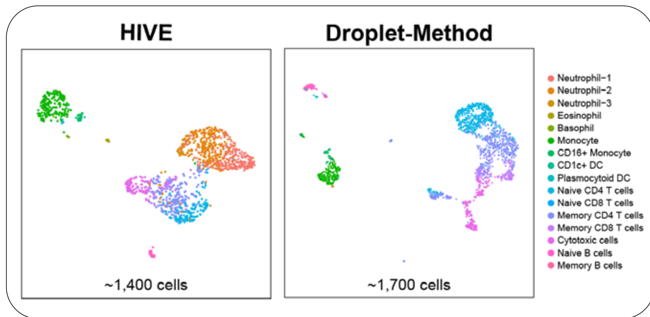


Figure 3. UMAP plot of high-quality single cells recovered from HIVE devices and droplet method, colored by cell type identity.

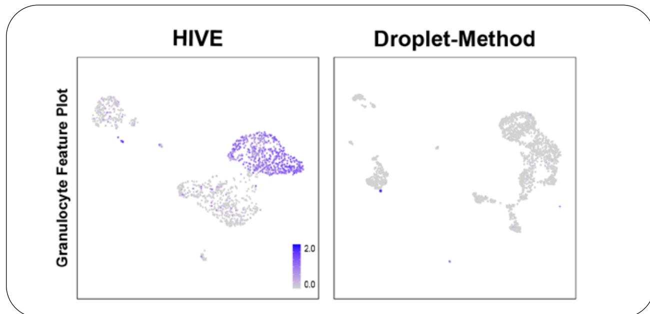


Figure 4. Feature plot showing the aggregate expression of granulocyte markers for neutrophils, eosinophils, and basophils - present in HIVE data but almost completely absent in droplet data.

In the data generated from the HIVE device, 44% percent of cells recovered from the sample were granulocytes. For the same sample loaded into the droplet platform, granulocytes comprise less than 0.5% of the recovered cells [Figure 5]. These percentages represent a dramatic reduction in the recovery of fragile cells using the droplet method.

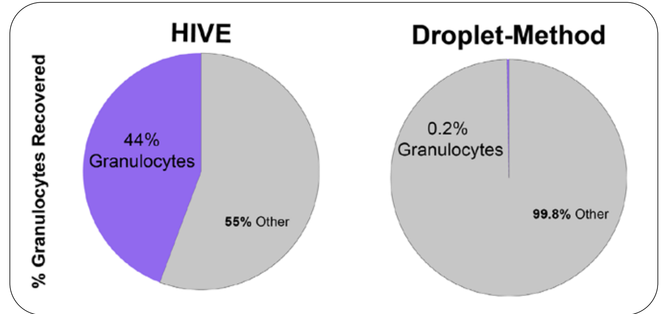


Figure 5. Pie chart showing percent granulocyte recovery between HIVE and droplet methods.

To determine if the granulocyte populations were recovered by the droplet method but filtered out as low-quality cells, the threshold for the droplet dataset was lowered to 50 genes and 100 transcripts. Annotation of this dataset shows recovery of low-quality neutrophil, eosinophil, and basophil populations [Figure 6]. The expression, however, of granulocyte marker genes are barely detectable with the droplet platform in comparison to the HIVE solution, which demonstrates robust expression of the same genes [Figure 7], suggesting that both the quality and biology of these fragile cells are distorted by the droplet method.

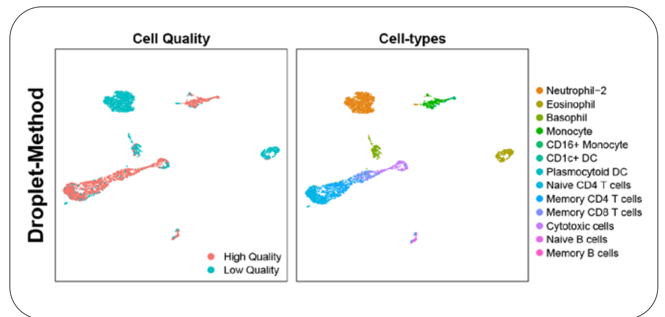


Figure 6. Reduced quality threshold for droplet data shows recovery of low quality granulocytes (thresholding = 50 genes, 100 transcripts).

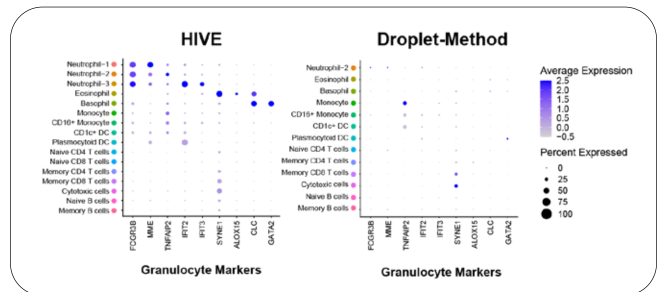


Figure 7. Dotplot showing the expression profile of specific granulocyte marker genes (columns) for each cell type (rows). Standard thresholding for HIVE data (> 400 genes and > 800 transcripts), low thresholding for the droplet-method (> 50 genes and >100 transcripts).

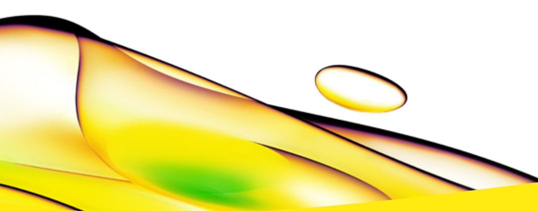
Conclusions

The results of this comparative study showcase the robust ability of the HIVE™ scRNAseq Solution to recover fragile granulocyte populations. In contrast, based on analysis parameters set, quality granulocytes are not recovered from the droplet method, calling into question whether the complete biology of a sample is being represented in the data. With the HIVE™ scRNAseq Solution, users can be confident in the ability to recover fragile cell types and preserve the biological profile of their samples. In addition, the HIVE™ scRNAseq Solution removes the logistical challenges associated with droplet-based technologies and enables improved experimental flexibility with:

- Sample storage between capture and processing workflows to promote multi-site and multi-time point studies
- No specialized equipment required
- Large sample loading volumes of up to 4 mL
- Potential to use stronger lysis buffers and customize reagents

References

1. Shen F, Li X, Li PC. Study of flow behaviors on single-cell manipulation and shear stress reduction in microfluidic chips using computational fluid dynamics simulations. Biomicrofluidics. 2014;8(1):014109. Published 2014 Feb 21. doi:10.1063/1.4866358



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