Consistent PBMC Transcriptomes Across Extended Processing Intervals with Asteria™ single cell RNA-Seq benchtop kit

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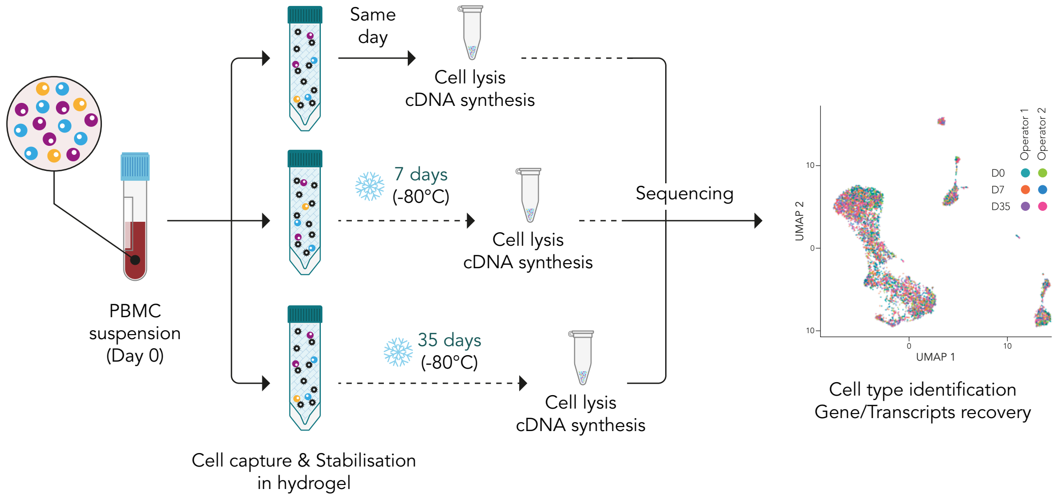
Summary

The timing of biological sample collection is frequently constrained by external factors such as clinical scheduling, multi-site study coordination, limited access to specialized facilities, or logistical delays in sample transport. In such contexts, it is essential to ensure that downstream single-cell RNA sequencing (scRNA-seq) data remain comparable and free from biases introduced by differences in collection-to-processing intervals. To model asynchronous sample collection, we took advantage of the early stopping point provided in the Asteria™ benchtop kit protocol, where captured cells are stabilized in a hydrogel, to investigate the extent to which samples remain comparable following storage for up to 35 days prior to library preparation.
By monitoring PBMC clustering, gene and transcript diversity, as well as established quality-associated signatures such as mitochondrial transcript proportion and apoptosis-related gene expression, our results indicate that samples can be stored for at least 35 days without detectable impact on these metrics. These findings support the use of extended storage following early stabilization, providing increased flexibility for experimental planning without compromising data comparability.

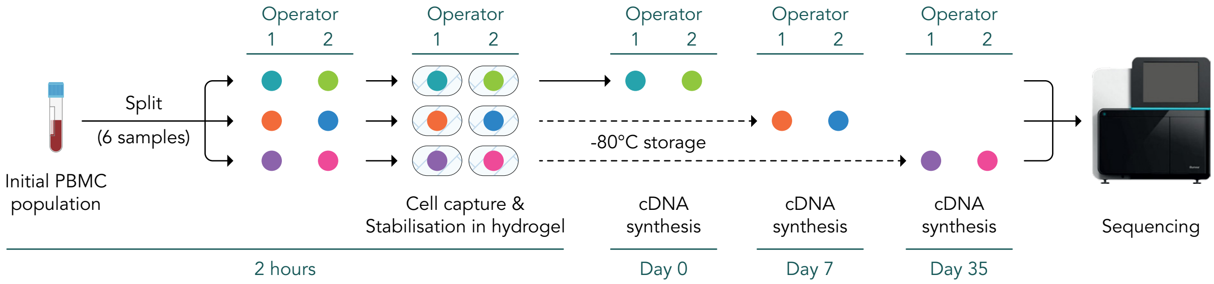
Graphical abstract



Experimental workflow. A single PBMC sample was divided into three aliquots to assess the impact of storage duration on downstream single-cell RNA-seq performance. One aliquot was processed immediately (Day 0), while the two others were stored at -80 °C for 7 and 35 days, respectively, prior to resuming cDNA library preparation. All samples were subsequently sequenced in the same run, and analyzed comparatively to evaluate potential differences in data quality.

Methods

Fresh Human Peripheral Blood Mononucleic Cells (PBMCs) were extracted from whole blood and split into six samples before treatment by two operators with three samples each. After isolation in the hydrogel within two hours (first stopping point of the Asteria™ protocol), samples were processed freshly (D0) or stored at -80°C for 7 (D7) or 35 (D35) days before resuming the protocol and generating barcoded cDNA. After library preparation (using the NEBNext Ultra II FS DNA Library Prep kit) and sequencing for the six samples, the obtained data was analyzed using Cytonaut™ (cytonaut-scipio.bio/login) to evaluate data quality ans transcriptomic profiles. All protocol steps are performed as described in the Asteria™ [1] and Cytonaut™ [2] user guides.



Results

Definition of cell type proportions is not altered following 7 and 35 days of storage.

To assess the impact of processing the samples at different time points (D0, D7 and D35), libraries were prepared simultaneously for all conditions, and samples were sequenced in the same run. The count matrix tables of all samples were merged and subjected to PCA and 2D projection on UMAP to evaluate how cells from each condition are distributed. Results shown in Fig.1A indicate that cells from all conditions are uniformly distributed across the UMAP, suggesting that neither the operator nor the storage duration introduced detectable bias in the data. The results are also presented as annotated cell type proportions on Fig.1B. Automated cell-wise classification identified the same cell types across all conditions, with comparable proportions (Fig.1B), indicating that sample storage in hydrogel for up to 35 days does not affect cell type identification.

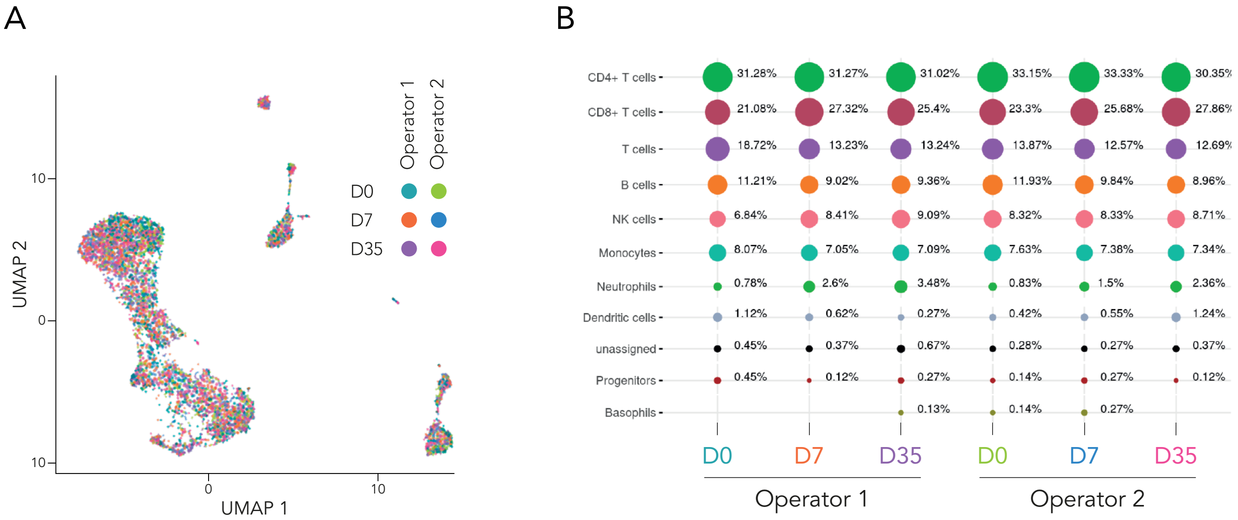


Figure 1

(**A**) Projection of scRNA-seq data on 2D (UMAP) of the tested conditions.
(**B**) Cell type proportions for each user at each timepoint following automatic annotation with the MonacoImmuneData[3] dataset.

Unaltered Transcriptomic Diversity and Stress Signatures Following Hydrogel Storage

Using sequencing data from the D0, D7, and D35 conditions, we assessed gene and transcript diversity for each sample to evaluate whether extended storage following stabilization impacts transcriptomic complexity (Fig.2). A slight decrease in the median number of transcripts per barcode and in the median number of genes per cell-associated barcode was observed for the stored conditions compared to the D0 control. However, both metrics remained within the expected performance range for PBMC samples processed with the Asteria™ kit, and no shifts in cell clustering based on gene expression profiles were detected.

To further assess transcriptome quality, we examined the proportion of mitochondrial transcripts, as well as the expression of genes associated with cold-shock response[4] and apoptosis[5] (Fig.3). Across conditions, the relative contribution of mitochondrial transcripts and the expression levels of apoptosis- and cold shock-related genes showed no substantial differences between stored samples (D7 and D35) and the reference condition (D0).

Overall, these results indicate that storage of stabilized samples for up to 35 days does not measurably impact transcriptomic quality or diversity compared to immediate processing.

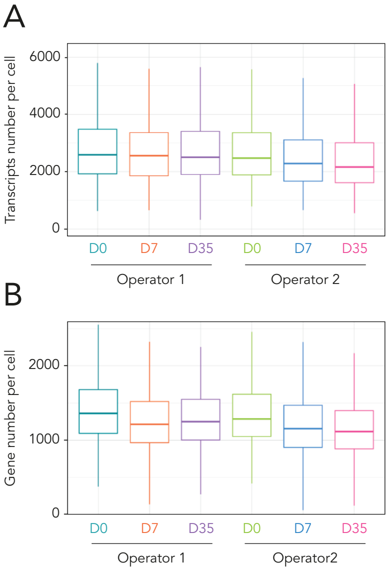


Figure 2

Box plots of the mean number of transcripts (**A**) and mean number of genes (**B**) per cell for each condition.

Figure 3

Box plots of the fractions of mitochondrial (**A**), cold shock-induced[4] (**B**) and apoptosis[5] (**C**) transcripts for each condition.

Conclusion

These results demonstrate that hydrogel-based stabilization preserves transcriptomic integrity and cell type composition for up to 35 days. This robustness enables decoupling of sample collection from processing, accommodating clinical scheduling constraints, multi-site study coordination, and limited access to specialized facilities, while ensuring consistent and comparable single-cell RNA-seq data across samples.

References

1. Asteria™ User guide v1.0, available on https://scipio.bio
2. Cytonaut™ User guide v1.5, available on https://scipio.bio
3. Monaco G. et al. (2019). Cell Rep. 26, 1627-1640.
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5.GSEA (2015), HALLMARK_APOPTOSIS, retrieved from http://www.gsea-msigdb.org/gsea/msigdb/human/geneset/HALLMARK_APOPTOSIS.html

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