

Recover complete biology in contrast to cryopreservation.

A case study with healthy human bone marrow.

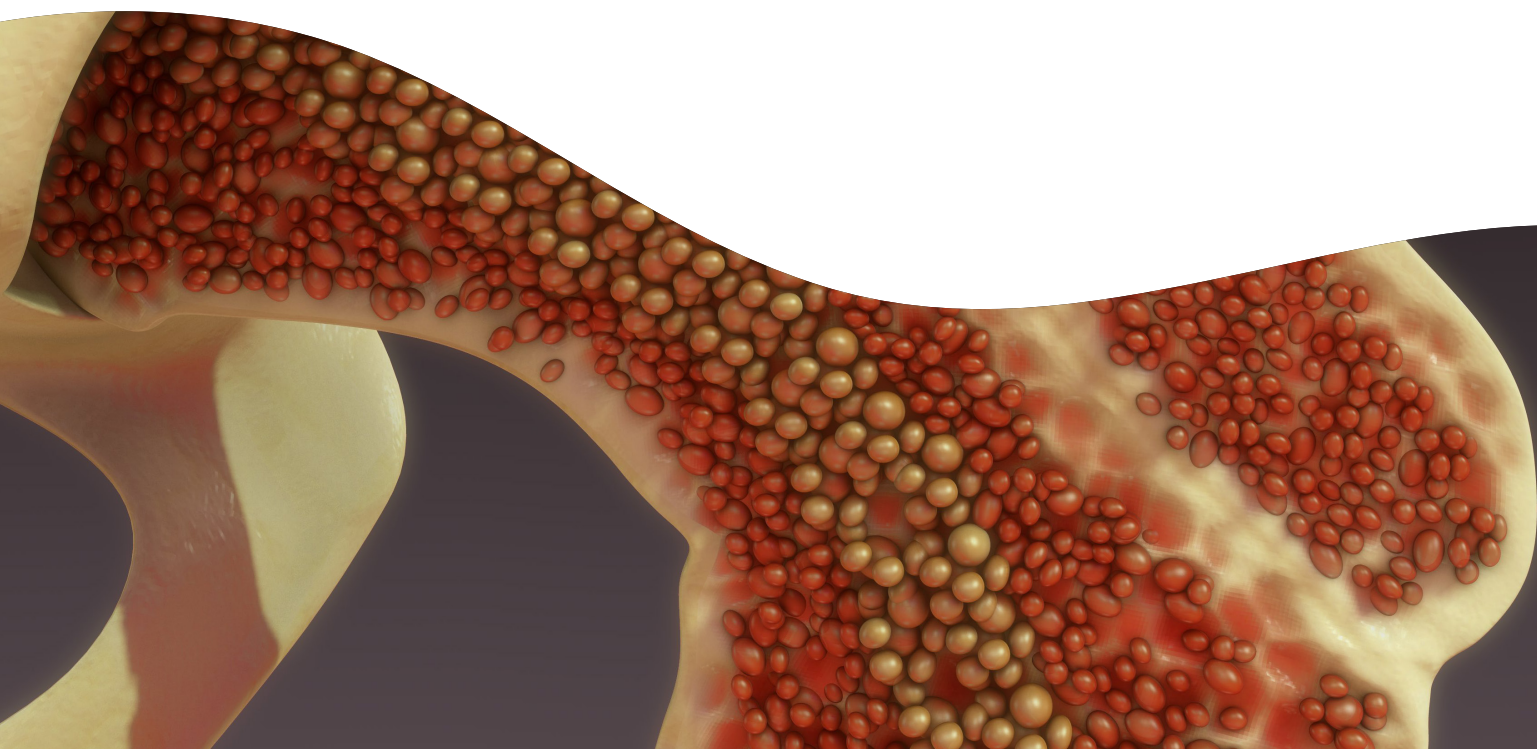
Key features

- Cryopreservation destroys cells that are critical to understanding biology of sample
- Integrated sample storage preserves cellular complexity
- Provides access to challenging sample types

Introduction

Single-cell RNA sequencing (scRNAseq) of minimally invasive liquid biopsies, such as blood, bone marrow, urine, and CSF enables researchers to gain valuable biological insights into tissue-resident diseases^{1,2}. Sites where these samples are collected often do not have the specialized equipment or trained personnel required by many scRNAseq technologies. Freezing and shipping single-cell samples to a centralized lab setting for processing into sequencing libraries allows for greater flexibility with multi-site and time course collections. Cryopreservation, a common storage method, can be irreproducible and cause poor cell viability and cell stress due to ice crystal formation during freezing, leading to poor data quality^{3,4}. The HIVE CLX™ scRNAseq Solution integrates sample storage and single-cell profiling into a single workflow. After cells are gently loaded into the HIVE collector, the Cell Preservation Solution locks the molecular profile at the time and site of collection. The quantity, quality, and biological complexity of cells stored in the HIVE device are comparable to freshly processed cells, so these important samples can be stored or shipped without compromise.

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Methods

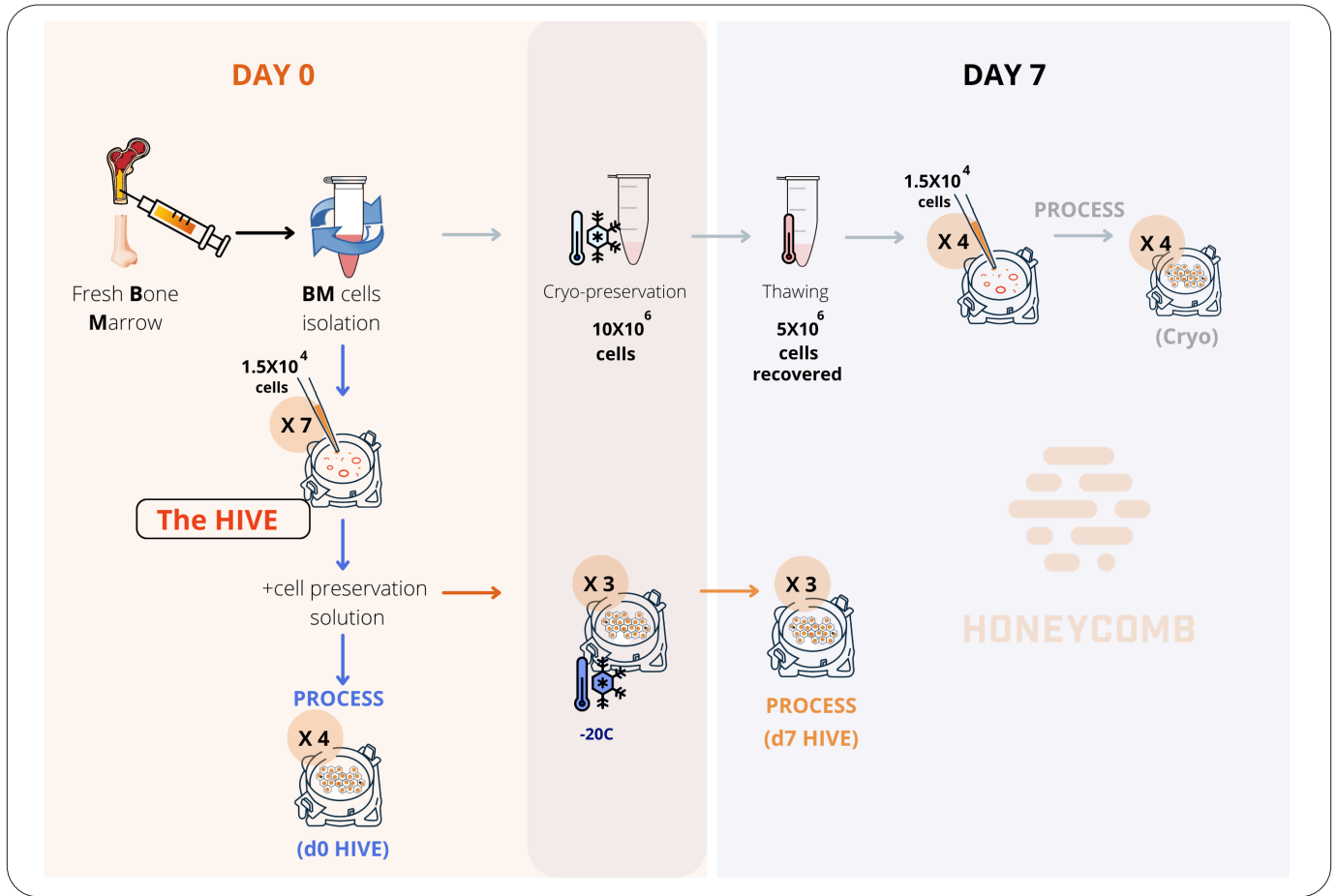


Figure 1. Experimental design

Fresh RUO grade bone marrow from a healthy human donor was used in this study (AllCells). After red blood cell depletion, seven HIVE collectors were loaded with $\sim 15,000$ cells per HIVE collector. Upon addition of Cell Preservation Solution, four HIVE collectors were processed immediately (d0) and three were frozen at -20°C for seven days prior to processing (d7). The remaining cells were cryopreserved and stored in liquid nitrogen for seven days before being loaded into four additional HIVE collectors and processed immediately (Cryo) [Figure 1].

Single-cell libraries were generated following the HIVE v1 protocol [workflow outlined in Figure 2], count matrix files were generated using BeeNet™ software, and data were analyzed using Seurat v4.0.5. Automated annotation of cell types was done using the healthy human bone marrow reference data set from DePasquale et al. 2021⁵.

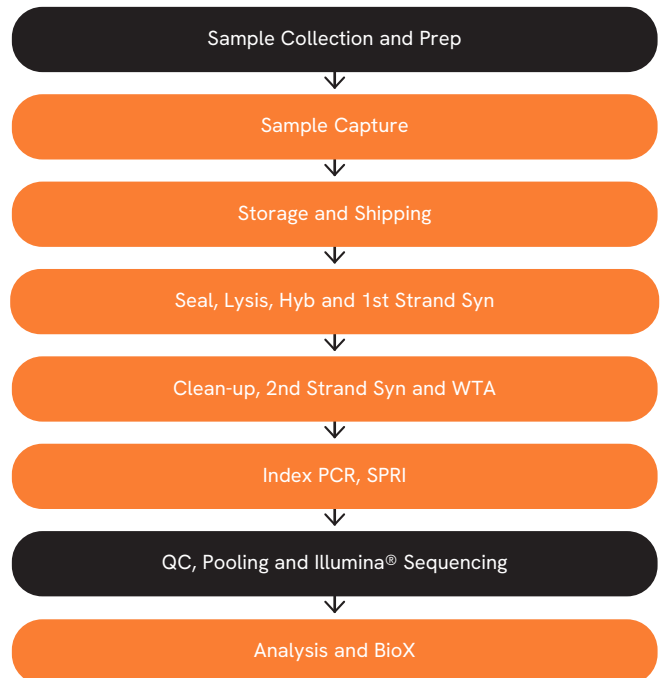


Figure 2. HIVE CLX scRNAseq workflow

Results

Cryopreservation resulted in significant cell loss during thawing; 10,000,000 cells were cryopreserved but only 5,150,000 cells were recovered, a loss of 48.5%. Furthermore, even though HIVE collectors were loaded with equal numbers of cells per condition, fewer cryopreserved cells were recovered with the HIVE device, likely due to

cell damage from cryopreservation [Figure 3A]. Other performance metrics, such as genes per cell and transcripts per cell, were similar between processing time points [Figures 3B, 3C]. Additionally, the cryopreserved cells recovered showed a higher percentage of mitochondrial reads, indicative of low-quality cells [Figure 3D].

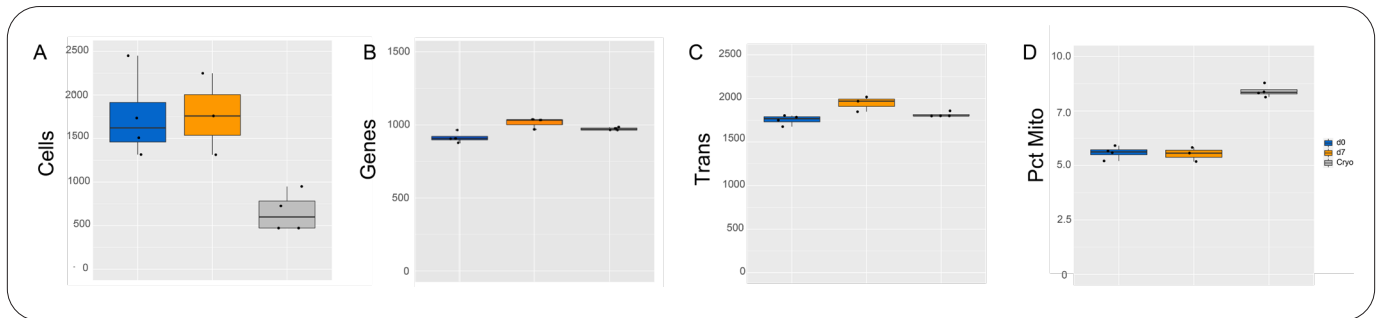


Figure 3. A) Number of high-quality cells recovered for each condition. B) Median unique genes per cell. C) Median unique transcripts per cell. D) Percentage of mitochondrial reads per sample

Bone marrow, the spongy tissue at the center of most bones, is the primary site of hematopoiesis, the production of new blood cells. Hematopoietic stem cells give rise to progenitor cells which then differentiate into platelets (thrombocytes), red blood cells (erythrocytes), and white blood cells (leukocytes). After thresholding and filtering for high quality single-cells, a UMAP plot of single cells from

all eleven HIVE devices was generated and colored by cell type [Figure 4A]. Twenty-three cell types were identified, including T cell, B cell, multiple stages of erythrocyte progenitors and fragile cell types, like neutrophils and eosinophils. Additionally, there was robust and specific expression of marker genes for each cell type [Figure 4B].

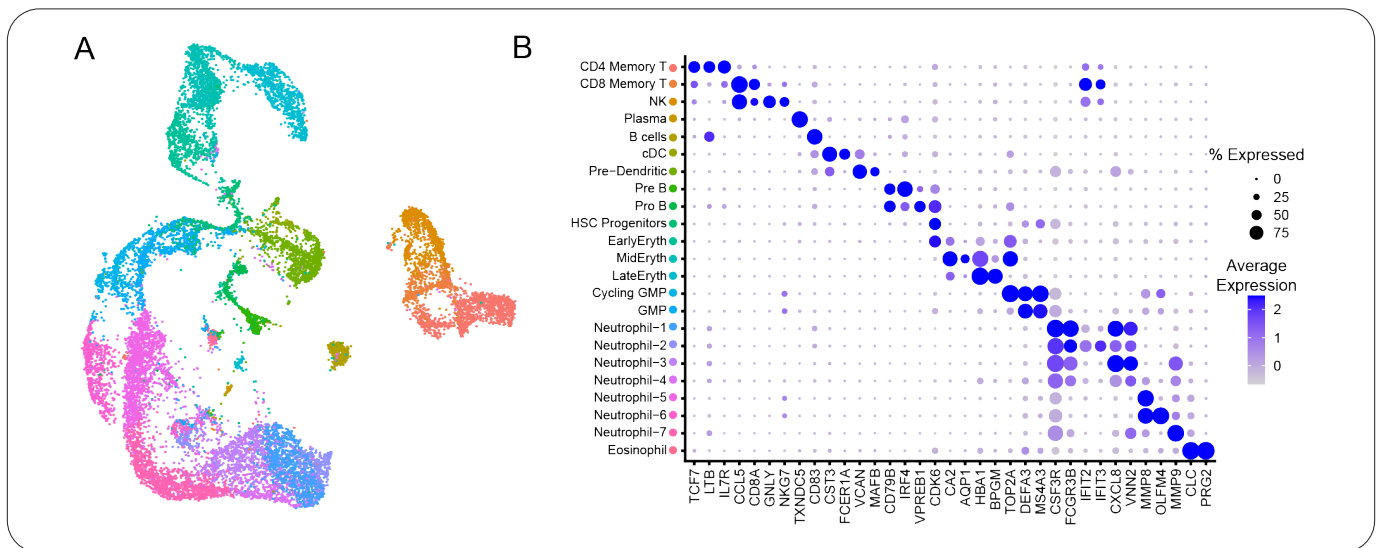


Figure 4. A) UMAP plot of high-quality single cells from eleven HIVE devices, colored by cell lineage. B) Dot plot showing the expression profile of specific marker genes (columns) for each cell type (rows)

When the cells are colored based on whether they were processed fresh (blue) or stored in a HIVE device (orange), the distribution of cells within each cell type is consistent; however, the cryopreserved samples (gray) did not retain all cell types [Figure 5A]. Approximately half of the cells recovered, from both the fresh and HIVE device stored samples, are cell types lost in the cryopreserved samples [Figure 5B]. Additionally, the relative frequency of each cell type is consistent between the fresh samples and those stored in the HIVE device for 7 days. In contrast,

the cryopreserved samples have partial or complete loss of some cell types, causing the remaining cell types to be over-represented [Figure 5C]. In particular, neutrophils, eosinophils, and granulocyte/macrophage progenitors (GMPs) were depleted in the cryopreserved samples but not the samples stored in a HIVE device [Figures 5B, 5C]. The cryopreserved samples also have a cluster of poor-quality neutrophils that are not present in the d0 and d7 samples [Figure 5A].

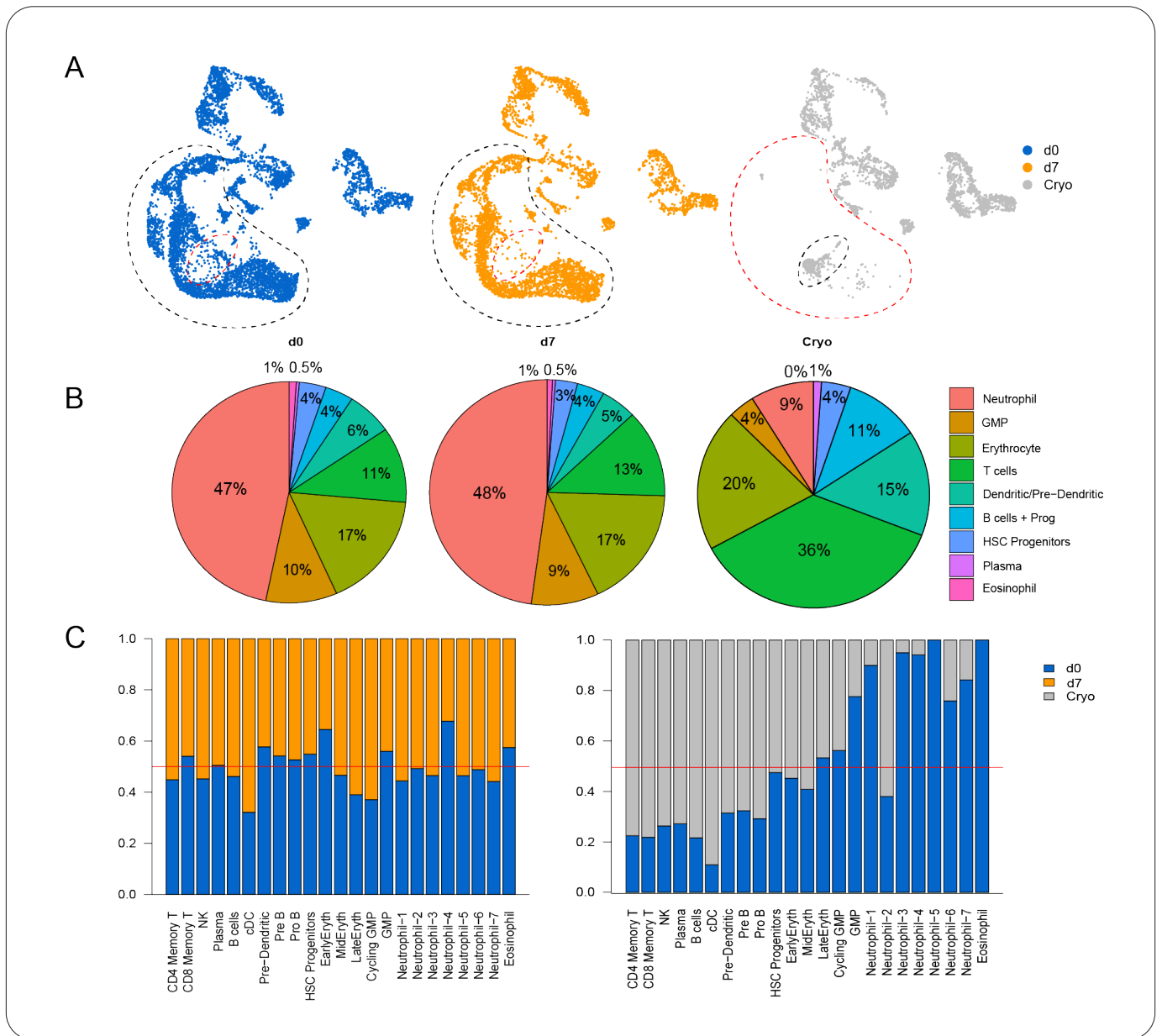


Figure 5. A) UMAP plots from HIVE devices processed fresh (blue), after storage in the HIVE collector for 7 days (orange), or after cryopreservation for 7 days (gray). B) Pie chart showing cell classes recovered for d0, d7, and cryopreserved samples C) Relative frequencies, normalized to 1, for each cell type for HIVE devices processed fresh (blue), after storage in the HIVE collector for 7 days (orange), or after cryopreservation for 7 days (gray)

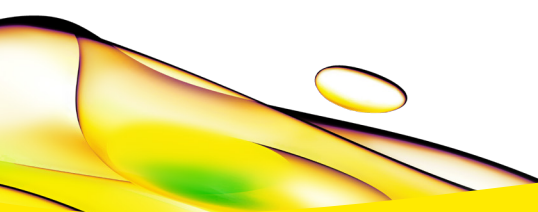
Conclusions

Overall, these results show that samples captured with the HIVE scRNAseq device maintain the quality and biological complexity of samples processed immediately, while cryopreservation results in loss of biologically important cell types due to incomplete cell recovery. The cell types destroyed by cryopreservation of bone marrow are critical to research in many areas, including leukemia, multiple myeloma, myelodysplastic syndromes, and rheumatoid arthritis. Single cell analysis of minimally invasive liquid biopsies can provide new knowledge within basic, translational, and clinical research.

The single cell storage integrated into the HIVE CLX scRNAseq Solution provides access to samples that have previously been challenging to collect without sacrificing biological information.

References

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