Stabilization of Human Cells for RNA Extraction Using Capillary-Mediated Vitrification Process

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PURPOSE

The cells of human, animal or bacteria, viruses, bodily fluids, or DNA/RNA, are collected, stored, and distributed throughout the globe for diagnostics, and research purpose. These biological samples typically need to be refrigerated or frozen to maintain the stability, and function of relevant biomarkers, including RNA and DNA. The requirement of cold storage adds significant logistical complexity and cost to these workflows. Development of new preservation technologies that aid ambient storage and delivery of these materials could eradicate the safety concerns associated with shipping materials on dry ice, minimize shipping costs by reducing package size and weight, eliminate the need for shipment by air, and reduce the risk of material loss both in storage and delivery due to temperature excursions.

OBJECTIVE(S)

To evaluate we innovated novel preservation method Capillary-Mediated Vitrification (CMV) [1-4] efficacy to preserve mammalian cells at ambient environments.

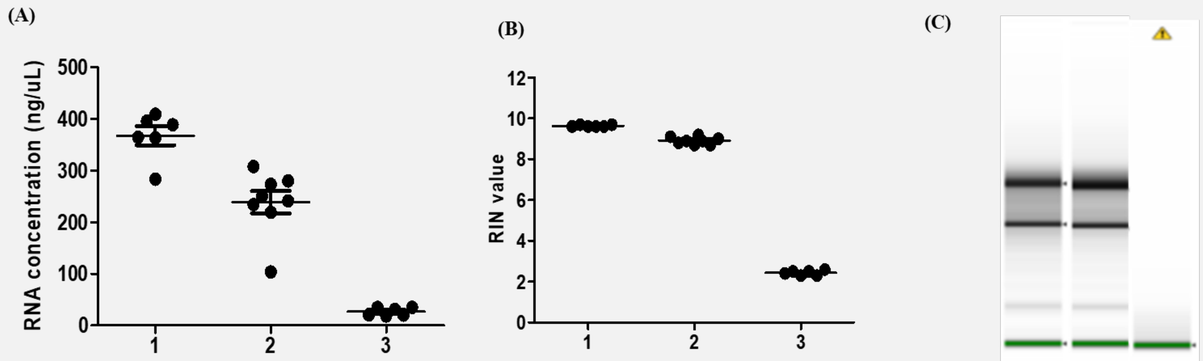
To assess whether the CMV process could be used as an alternative to frozen storage to maintain cellular RNA, which can be used for sequencing and biorepository applications.

METHOD(S)

The CMV process was executed by mixing 5x10^6 Jurkat cells with an excipient buffer and adding the mixture to a porous scaffold. The loaded scaffolds were placed into our vitrification chamber and dried for 30 min. After drying, the CMV-stabilized Jurkat cells were packed in Mylar pouches were stored at 55°C for 3 days. Cells resuspended in buffer were stored at 55°C for 3 days as a negative control. Freshly cultured Jurkat cells were used as positive control.

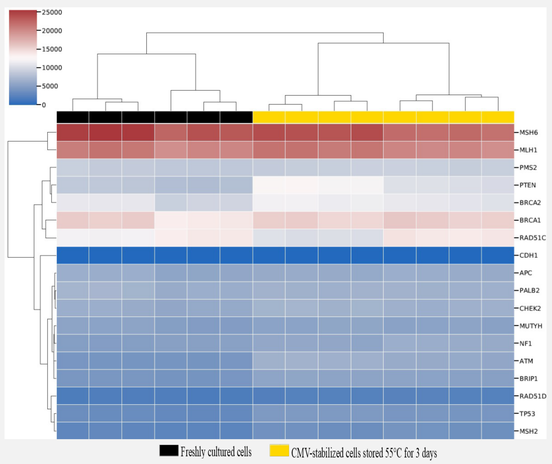
Following storage, total RNA was isolated, and the nucleic acid concentration was analyzed by Nanodrop. The RNA extracted from positive control and CMV-stabilized Jurkat cells was utilized to screen 18-tumor suppressor genes transcripts per million, and percent spliced in index profile using RNA-sequence analyses.

RESULT(S)

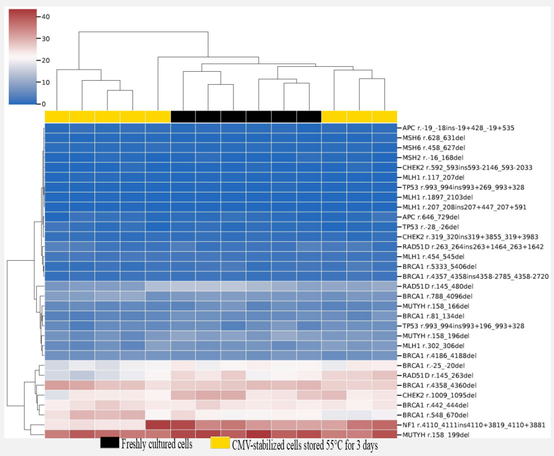


1: Freshly cultured Jurkat cells; 2: CMV-stabilized cells stored at 55°C for 3 days; and 3: Cells stored in buffer at 55°C for 3 days

Quantity and quality of total RNA isolated from CMV-stabilized and stressed, stressed cells stored in PBS, and positive control Jurkat cells. (A) RNA yield per 5 million cells; (B) Calculated RIN values; and (C) Representative RIN electrophoretic analysis of RNA integrity. Data represent the mean value of six to eight samples ± SEM. (CMV: Capillary-mediated vitrification, RNA: Ribonucleic acid, RIN: RNA integrity number, SEM: Standard error mean)



Expression heatmap of 18-tumor suppressor genes transcripts per million reads for CMV-stabilized and stressed, and freshly cultured Jurkat cells (n= 6 to 8). (CMV: Capillary-mediated vitrification).
CMV-stabilized Jurkat cells have similar gene transcripts to freshly cultured cells.



Percent spliced in index heatmap of 18-tumor suppressor genes for CMV-stabilized and stressed, and freshly cultured Jurkat cells (n= 6 to 8). (CMV: Capillary-mediated vitrification).
CMV-stabilized Jurkat cells have similar percent spliced in index to freshly cultured cells.

CONCLUSION(S)

We have demonstrated that the CMV technology can be used to stabilize mammalian cells for the purpose of nucleic acid extraction.

The use of the CMV process can significantly enhance the thermostability of these materials and may enable both storage and shipment at ambient temperature.

The CMV stabilized Jurkat cells used in this study were sufficiently stable to enable the extraction of the cellular RNA and RNA-seq analysis despite storage at 55 °C for up to three days.

We believe this process will be applicable to a broad range of cell lines, biospecimen matrices, and analytical methodologies.

Preparation and use of CMV samples

REFERENCE

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