

Effects of Cryopreservation on P450 Oxidation and Phase II Conjugation Activities in Human Hepatocytes:

Results with Hepatocytes from 30 Donors

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Introduction

- Human hepatocytes represent an important tool for the evaluation of human drug properties.
- Technologies for the effective isolation, culturing, and cryopreservation of highly viable human hepatocytes have been developed in our laboratory.
- In this study, the drug metabolizing enzyme activities of cryopreserved and freshly isolated human hepatocytes are compared.

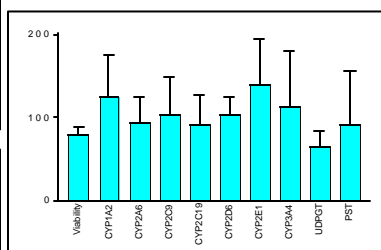
Methods

- The fresh hepatocytes were isolated from 30 different fresh human livers, using a 2-step collagenase perfusion procedure of Li et al (1992).
- The hepatocytes were assayed for drug metabolizing capacity as freshly isolated cells. Cells from the same preparation were then cryopreserved using a proprietary method. The cells were assayed after storage in liquid nitrogen for over 1 week.
- To evaluate drug metabolizing enzyme activities, CYP450 isoform-specific substrates and umbelliferone were incubated with the fresh and cryopreserved cells at 37 °C/5% CO₂ for 2 hours. Metabolites produced during this time period were quantified using HPLC.
- Data were plotted as activity after cryopreservation versus activity before cryopreservation.

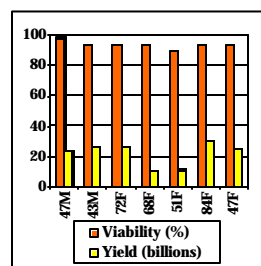
Donor Demographics

	Number of Donors		Number of Donors		Number of Donors	
Sex	Male	15	Female	15		
Ethnicity	White	26	Black	2	Hispanic	2
Age (year)	0-25	3	26-50	12	50-90	15
Smoker	Yes	16	No	14		
Alcohol Use	Never/Unknown	18	Socially	11	Abusive	1

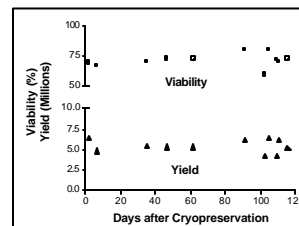
Viability and Drug Metabolism Activities of Cryopreserved Human Hepatocytes (Expressed as Percent of Fresh Hepatocytes)



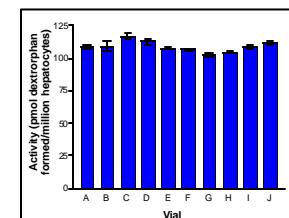
Human Hepatocyte Isolation: Viability and Yield



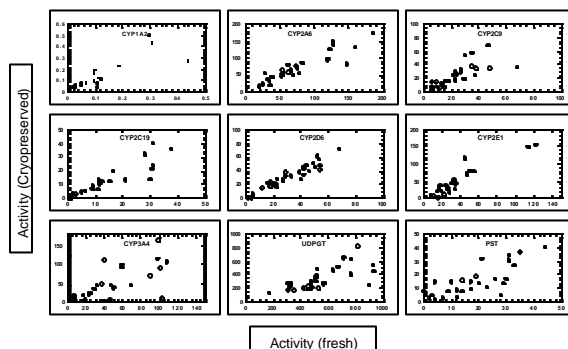
Viability and Yield as a Function of Cryopreservation Duration



Cryopreserved Human Hepatocytes: Vial-to-Vial Differences in CYP2D6 Activity



Fresh vs. Cryopreserved Human Hepatocytes Drug Metabolizing Enzyme Activities



Summary

- Cryopreservation of human hepatocytes maintains viability, yield, and metabolic activities as compared to freshly isolated human hepatocytes. All activities measured in the cryopreserved hepatocytes averaged near 100% of that of the freshly isolated cells, with the exception of umbelliferone glucuronidation (66 ± 18%) with very little vial-to-vial differences.
- Cryopreservation increases both convenience and efficiency by allowing freedom of experimental scheduling, performance of multiple experiments on hepatocytes from the same donors, and knowledge of the metabolic activities of the hepatocytes being used.

References

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