Liquidus Tracking - a new cryopreservation method for mammalian cells in three dimensional culture – useful for a biomass in a Bioartificial Liver

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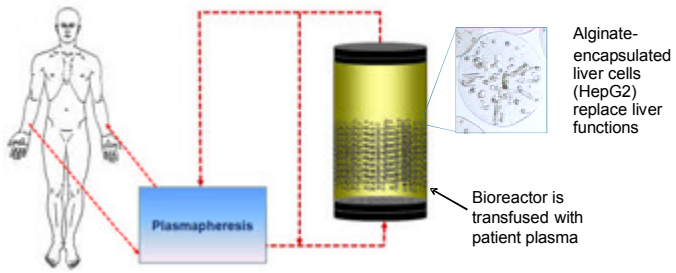
Introduction

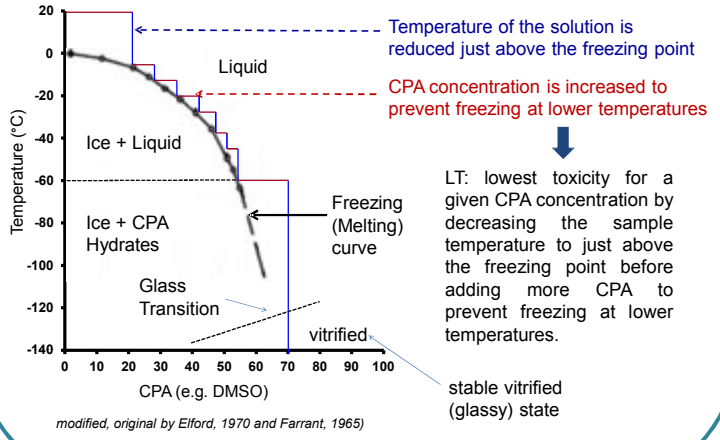
A bioartificial liver device is a short term solution for patients with acute liver failure until the liver has recovered or a liver transplant is available.



For clinical application large quantities of cells should be available immediately necessitating cryo-banking. Cryopreserving large volumes results in increased ice formation and increased cell death. By using high concentrations of cryoprotectants (CPAs) at low temperatures ice formation can be prevented; this is known as vitrification. However, high amounts of CPAs are toxic to the cells. Liquidus tracking is a new cryopreservation method that provides a vitrification process at reduced CPA toxicity.

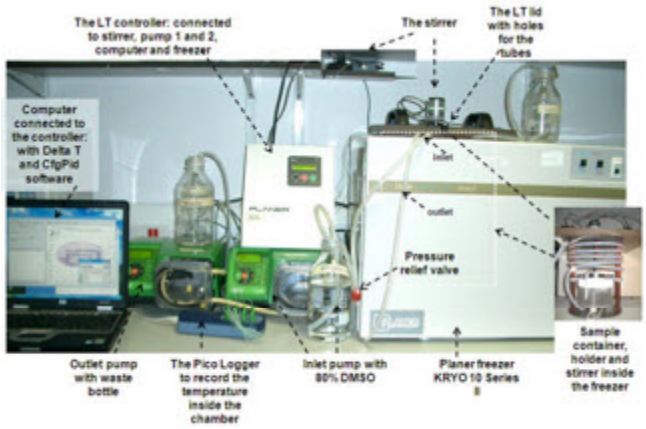
The principle of Liquidus Tracking (LT)

CPA toxicity is reduced at lower temperatures due to less metabolic cell activity.



modified, original by Elford, 1970 and Farrant, 1965)

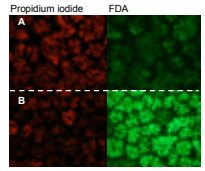
2.- Setting up the Liquidus Tracker



Alginate encapsulated liver cells are added to the sample container which is placed inside the controlled rate freezer of the Planer Liquidus Tracker. The CPA concentration of the sample is increased by pumping a highly concentrated CPA solution into the container while the freezer cools down. A stirring system mixes the CPA concentration to achieve a homogeneous concentration (developed by Planer and David Pegg).

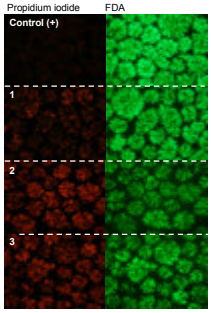
3. - Reducing CPA toxicity by using different CPA combinations

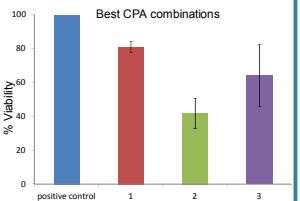
To further increase cell viability, a low-toxicity CPA solution was developed with the requirement of low viscosity so that it may be used within the Liquidus Tracker. In total 29 different combinations of ethylene glycol (EG), propylene glycol (PG), DMSO, glucose and methanol were tested for toxicity using fluorescein diacetate (FDA) and propidium iodide staining.



	DMSO, EG, PG	methanol	glucose
A	34% DMSO, 9% EG, 9%PG	9%	–
B	34% DMSO, 9% EG, 9%PG	4.5%	4.5%

	Viability (%)
A	7.45
B	83.72





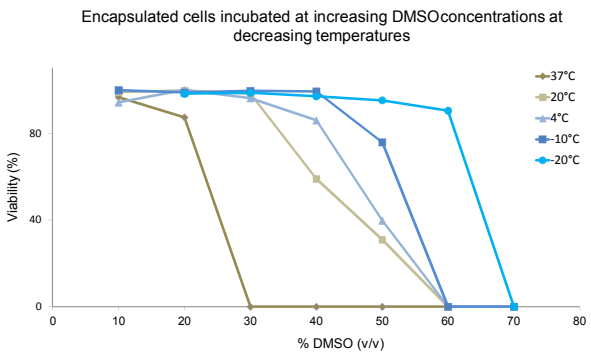
Solution	%DMSO	%EG	%methanol	%glucose
1	34	17	0	9
2	43	9	0	9
3	17	26	9	9

Replacing 4.5% methanol with 4.5% glucose increases viability (A, 76%). Replacing DMSO with EG increases viability (Solution 1). Solution 3 shows that methanol can be added to decrease viscosity while maintaining high viability- applies only if DMSO and PG concentration is low.

Results

1.-Proof of principle

CPA toxicity (DMSO) is reduced at lower temperatures:



Conclusion

- Cryoprotectant toxicity is reduced at lower temperatures.
- The addition of glucose and partially replacing DMSO with ethylene glycol reduces CPA toxicity.
- Methanol can be used to decrease viscosity but DMSO and PG concentration needs to be kept low to maintain high cell viability.

A low-toxicity CPA solution in combination with Liquidus Tracking should result in sufficiently high cell viability and function in a biomass for use in a bioartificial liver.

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