The effect of cryopreservation on cell viability, recovery and proliferation

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Introduction

The ability to freeze, store and recover cells makes cryopreservation an attractive process to meet the supply demands for high throughput analysis in drug screening programmes. Despite this many laboratories perform cryopreservation using suboptimal procedures which can severely affect cell performance in downstream applications.

This study aims to understand the impact that cryopreservation has on cell behaviour by examining the effect of the cryoprotectant solution (6 solutions, see Table 1), the length of storage at -80oC (6, 24 and 72 hours) and the length of storage in liquid nitrogen (1 or 10 weeks) on the viability, recovery and proliferation of a cell line (HepG2) commonly used in drug toxicity assays.

Results

Cell viability following cryopreservation

	Cryoprotectant solution		
	Components		
	Glycerol	DMSO	Serum
CryoMaxx™		10%	90%
CryoStor™		10%	0%
DMSO low serum		90%	10%
DMSO high serum		10%	90%
Glycerol low serum	90%		10%
Glycerol high serum	10%		90%

Table 1 – Composition of the six different cryopreservation solutions

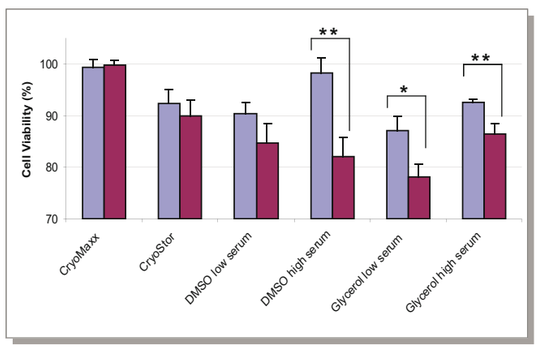

Figure 1 – Impact of liquid nitrogen storage on cell viability in the six cryopreservation solutions. Longer storage in liquid nitrogen (10 weeks) leads to significantly lower viability in cells stored in DMSO-high serum (**p<0.01), glycerol-low serum (*p<0.05) and glycerol high serum (**p<0.01).

Analysis of cell recovery

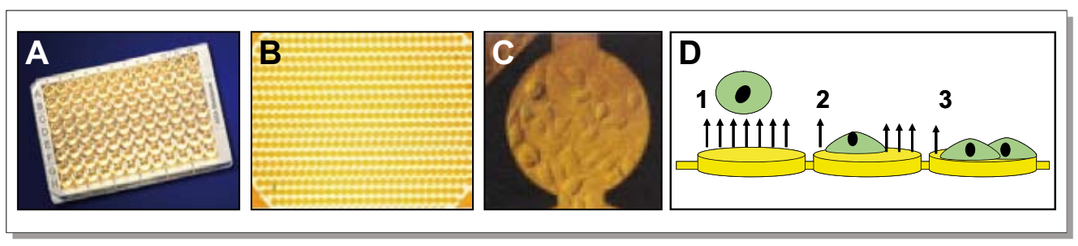

Figure 2 – Analysis of cell recovery following cryopreservation using impedance measurements. A gold electrode array on the base of the multiwall chamber (A and B) is used to measure the impedance caused by adhered cells (C). As cells divide the impedance increases allowing real time measurement of proliferation rates (D).

Cell recovery following cryopreservation

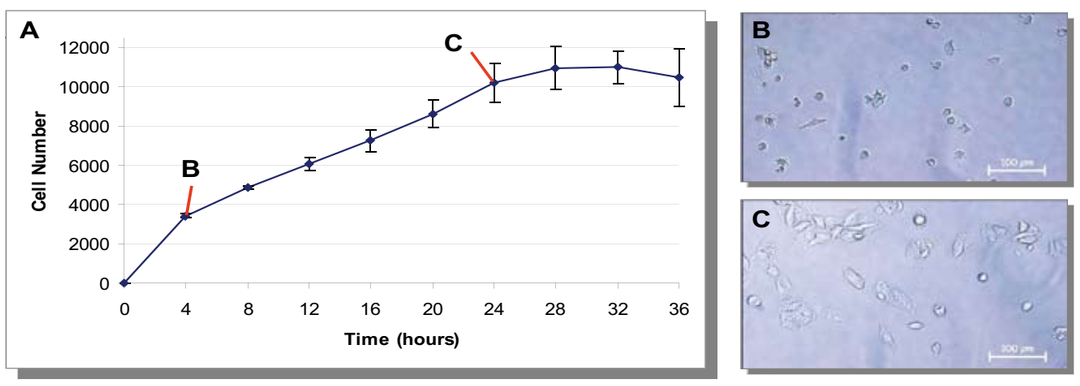

Figure 3 – The process of cell recovery analysed using impedance measurements on Mytomycin-C treated cells (A). Two key events occur during cell recovery, an initial phase of cell attachment over the first 4 hours (B) following by a spreading phase over the next 20 hours (C)

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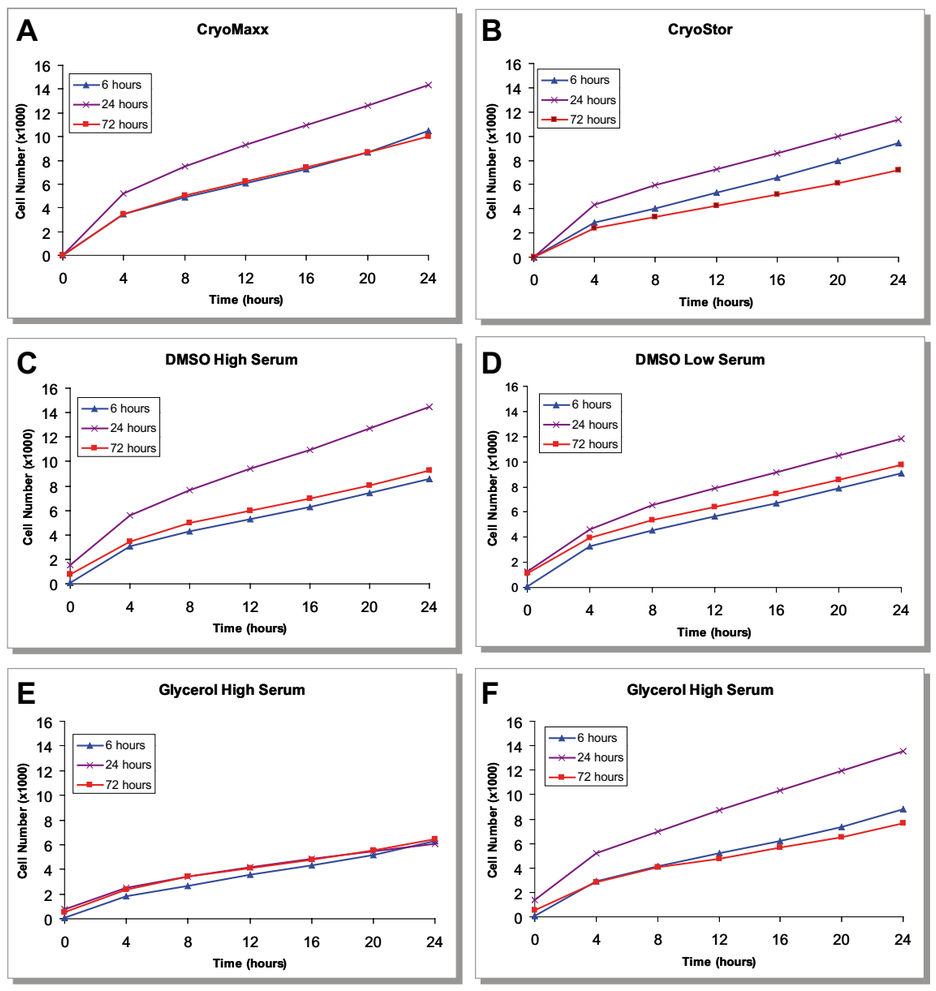

Figure 4 – Storage at -80ºC has a large impact on cell recovery. With the exception of cells cryopreserved in glycerol low serum (E) the highest recovery of cells was achieved following 24 hours storage at -80ºC. Storage of cells at -80ºC for shorter (6 hours) or longer (72 hours) periods resulted in 15%-35% lower recovery rates depending on the cryopreservation solution used.

Cell proliferation following cryopreservation

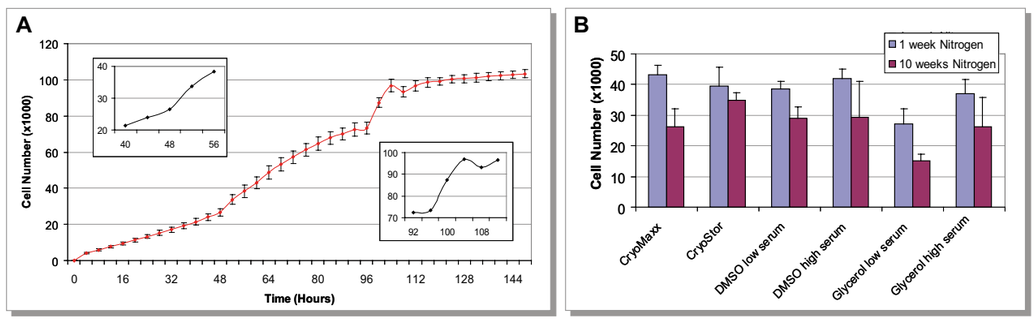

Figure 5 – Cell proliferation following cryopreservation. After 24 hours cells enter a linear growth phase (A) of ~10% every 4 hours. When the cells are fed after 48 hours the proliferation rate briefly increase to over 20% (upper box in A) before stabilising and reducing to <3% as the cells use up the nutrients in the media. The cells again undergo rapid proliferation (~16% over 4 hours) upon feeding after 96 hours (lower box in A). Cell proliferation rates after 72 hours are also affected by storage times in liquid nitrogen (B) with significantly lower proliferation rates measured in cells stored in liquid nitrogen for 10 weeks and cryopreserved in CryoMaxx, DMSO-low serum and glycerol-low serum (p<0.05).

Conclusions

- Cell viability following recovery from cryopreservation is significantly affected by cryopreservation solution and the storage time in liquid nitrogen.
- Cell recovery following cryopreservation takes ~24 hours and is significantly affected by amount of time cells are stores at -80ºC and in liquid nitrogen.
- Following recovery cells enter a linear proliferative phase which is influenced by the nutrient content of the growth media.
- The choice of cryopreservation solution used to freeze cells has a significant impact on the proliferative potential of the cells following recovery.
- Long term storage in liquid nitrogen significantly affects cell proliferation.

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