Nunc Cryobank Storage System: Viability of Mammalian Cells Recovered from Peripheral and Central Rack Positions

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Introduction

The Nunc Cryobank storage system of 96 cryovials in an SBS-footprint rack benefits from a dense storage format for optimized utilization of freezer space and easy handling of many cryovials at a time, for example, for centrifugation of thawed vials.

Preserving samples in a dense storage format could possibly give rise to variability in the viability of retrieved cells, if vials placed in the centre of the rack were insulated by the surrounding vials. We cryopreserved two primary cell types and two cell lines in the Cryobank system and compared cell viability in thawed vials placed in the peripheral positions of the rack and in vials placed in the central positions of the rack.

Centrifugation of thawed vials may be used for quick removal of the cryogenic freezing agent in order to get a high viability of retrieved cell populations. We tested whether the Cryobank system could withstand repeated and extensive centrifugation.

Cell viability

Determination of cell viability for HUVEC, CHO and MDCK (Figure 1.B, C and D) showed that viability of retrieved cells was not compromised, irrespective of the vials being placed in peripheral or central positions of the storage rack. For the primary cell type, HDFa (Figure 1.E), a significant variability in viability of retrieved cells in vials in peripheral and central positions was observed. In order to investigate if the variability was due to a decreased retrieval of viable cells in vials placed in central positions or due to an improved retrieval of viable cells in vials placed in peripheral positions, we compared cell viability of the HDFa cells in Cryobank vials with cell viability in standard Cryovials. The results show that the viability of retrieved cells in Cryovials is identical to viability of retrieved cells in vials placed in central positions (Cryovials 90.6% ± 1.8, Cryobank 90.6% ± 0.8, data presented as means ± SEM). It is, therefore, concluded that the variability observed using HDFa in the Cryobank system is due to improved retrieval of viable cells in vials placed in peripheral positions rather than lower retrieval of viable cells from vials in the central positions.

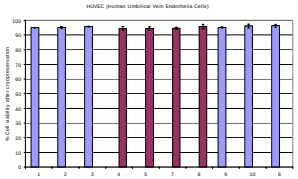

Figure 1.B Determination of cell viability for HUVEC revealed no significant difference after cryopreservation in peripheral or central positions of the rack. Bars represent means ± SD (n=3).

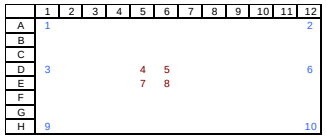

Figure 1.A Cell viability measured on vials placed in peripheral positions of the rack is shown by blue colour and vials placed in central positions are shown in red. The remaining positions in the rack were occupied with vials filled with culture medium only. This set-up was repeated 3 times for each cell type.

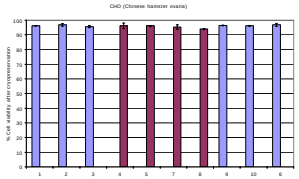

Figure 1.C Determination of cell viability for CHO revealed no significant difference after cryopreservation in peripheral or central positions of the rack. Bars represent means ± SD (n=3).

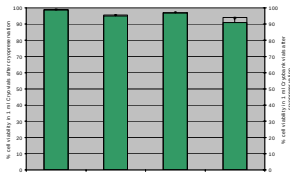

Figure 2. Determination of cell viability in Cryobank vials (open bars) and NUNC 1 mL Cryovials (green bars). Data is presented as means + SEM. For MDCK, HUVEC and CHO, there was no pronounced difference in cell viability after cryopreservation in the Cryobank system or Cryovials. For HDFa a significantly higher cell viability was observed after cryopreservation in the Cryobank system.

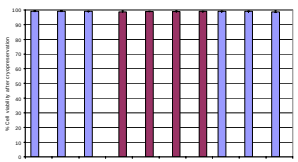

Figure 1.D Determination of cell viability for MDCK revealed no significant difference after cryopreservation in peripheral or central positions of the rack. Bars represent means ± SD (n=3).

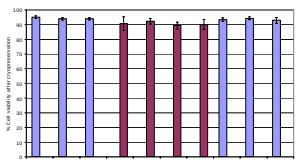

Figure 1.E Determination of cell viability for HDFa cells revealed a higher variability of cells frozen in and retrieved from peripheral positions of the rack (P<0.0002). Bars represent means ± SD (n=3).

Centrifugation of the Cryobank system

The Cryobank vials and rack was investigated for appearance of stress-lines or other deformities after centrifugation. Twenty vials first centrifuged at 300*g* were exposed to centrifugation at 500*g*, 1200*g* and finally 2000*g*. No observations of either stress lines or deformities of the vials were observed.

Centrifugation period	*G-value*	*Stress lines/deformities*
3 x 10 minutes	300	None
3 x 10 minutes	500	None
3 x 10 minutes	1200	None
3 x 10 minutes	2000	None

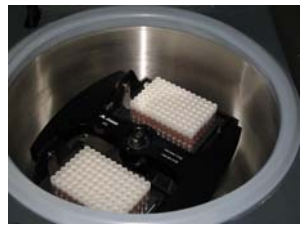

Figure 3. The Cryobank vials and were exposed to repeated and extensive centrifugation. No observations of either stress lines or deformities of the vials were observed.

Conclusion

Determination of cell viability for two primary cell types and two cell lines cryopreservated in the Nunc Cryobank storage system shows that viability of retrieved cells was not compromised due to the dense format of the Nunc Cryobank storage system. An improved retrieval of viable HDFa from Cryobank vials placed in peripheral positions was observed and might be because the Cryobank vials are slimmer than standard Cryovials, thus allowing a quicker freezing of the cell suspension.

The SBS-footprint of the Cryobank rack allows it to be subjected to centrifugation. Figure 3 shows that Cryobank vials and rack can tolerate extensive centrifugation, without being damaged. This feature can ensure quick removal of the cryogenic freezing agent and support protocols in achieving a high level of viable cells.

Materials and Methods

Cells:

CHO (Chinese Hamster Ovary) cell line, **MDCK** (Madin-Darby Canine Kidney) cell line, **HUVEC** (Human umbelica vein endothelia cells) primary cell, **HDFa** (Human derived fibroblast from adult skin) primary cell were cultured according to standard protocols

Cell Viability:

Cells were grown to 75-80% of confluence, harvested with Trypsin-EDTA (Cambrex BE17-161E), and dispensed in Cryobank vials.Six vials placed in the peripheral positions of the rack and 4 vials positioned in the central positions of the rack (scheme 1) were filled with 1.0 mL of cell suspension (containing 1.0 x 10^6 cells and 7.5% v/v DMSO). The remaining 86 vials in the rack were filled with 1.0 mL culture medium supplemented with 7.5 % DMSO. The rack was placed in a box of expanded polystyrene, and incubated over night at –80°C (an approximate cooling rate of 1°C/min was attempted by using this method). The rack was then transferred to the vapour phase of a liquid-nitrogen freezer and incubated over night. For determining the cell viability, the rack was transferred to –80°C, and three vials were assayed at a time. The vials were thawed at 37°C, and cell viability was immediately determined using a NucleoCount cell counter (Chemometec, Denmark). For determination of the total cell number the thawed cell suspension was diluted 1.1 with medium and 300 µL cell suspension was mixed with equal volume of lysis buffer and vortexed. 300 µL stabilisation buffer was added to the cell lysate and vortexed. This cell lysate was loaded into a NucleoCasette and placed into the NucleoCounter for analysis. The NucleoCasette contains a fluorescent propidium iodide, which intercalates with DNA in the cell nuclei. During analysis the fluorescent signal is counted and correlated to total cell count. For determination of non-viable cells, 300 µL diluted cell suspension was loaded directly into the NucleoCasette and analysed.

The cell viability was calculated by: % cell viability = (total cell number – number of non-viable cells)/total cell number x 100.

Comparisons of cell viability for the 4 cell types were performed using an independent, unpaired t-test and analysis of variance (ANOVA), and a significance level of 0.05.

Centrifugation Test:

Twenty Cryobank vials were filled with 1.0 mL culture medium (E-MEM with 10% FBS) supplemented with 7.5% DMSO, and then frozen and thawed as described above. The thawed vials were centrifuged in the rack: three times for 10 min at a stress of 300 – 2000*g*. The vials were visually inspected for deformities and appearance of stress lines after each 10-min centrifugation step. In order to expose the vials to as much stress as possible, vials centrifuged at 300*g* were also centrifuged at 500*g*, 1200*g* and 2000*g*.