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# Comparison of Unisol With Euro-Collins Solution as a Vehicle Solution for Cryoprotectants

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A VARIETY of factors are known to influence the survival of cells during cryopreservation, but the role of the vehicle solution for the cryoprotective agents (CPAs) is often overlooked. It is generally assumed that conventional culture media used to nurture cells at physiologic temperatures will also provide a suitable medium for exposure at low temperatures. However, it is now well established in tissue and organ preservation that maintenance of the ionic and hydraulic balance in cells during hypothermia can be better controlled by using solutions designed to physically restrict these temperature-induced imbalances.[1,2]

**Table 1. Comparative Solution Formulations**

| Components (mmol/L) | EC | Unisol-CV |
|---|---|---|
| Ionic | | |
| $Na^+$ | 10.0 | 60.0 |
| $K^+$ | 115.0 | 70.0 |
| $Ca^{++}$ | — | 0.05 |
| $Mg^{++}$ | — | 15.0 |
| $Cl^-$ | 15.0 | 30.1 |
| pH buffers | | |
| $H_2PO_4^-$ | 15.0 | — |
| $HPO_4^{2-}$ | 42.5 | — |
| $HCO_3^-$ | 10.0 | 5.0 |
| HEPES | — | 35.0 |
| Impermeants | | |
| Lactobionate$^-$ | — | 30.0 |
| Sucrose | — | 15.0 |
| Mannitol | — | 25.0 |
| Glucose | 194.0 | 5.0 |
| Gluconate | — | 70.0 |
| Colloids | | |
| Dextran 40 | — | 6% |
| Pharmacologics | | |
| Adenosine | — | 2.0 |
| Glutathione | — | 3.0 |
| Osmolality (mOsm/kg) | 375 | 350 |
| pH | | 7.6 |

EC (Euro-Collins solution; Unisol-CV, Unisol cryoprotectant vehicle, phosphate-free UHK).

On this basis, we have formulated a new hypothermic preservation solution (designated Unisol). A cryoprotectant vehicle version of Unisol (Unisol-CV) was compared with Euro-Collins (EC), an established organ preservation solution that has previously been used as a vehicle solution for CPAs (Table 1).[3]

## MATERIALS AND METHODS

Two cell types, a smooth cell line derived from rat thoracic aorta and an endothelial cell line derived from bovine corneal endothelium, were prepared as adherent cells in 96-well microtiter plates and exposed in a stepwise manner at 5-minute intervals to a range of concentrations of either dimethylsulfoxide (DMSO) or 1,2-propanediol (PD), for a final exposure of 10 minutes at 4°C. Elution of the CPAs was accomplished using mannitol as an osmotic buffer at 4°C. Additional groups of cells were treated similarly except they were frozen and thawed in the presence of the various preservation solution/CPA combinations. After rewarming to 37°C and replacement of the culture medium (DMEM/10% fetal calf serum), all groups of cells were assessed for metabolic activity using the nontoxic indicator Alamar Blue (Trek Diagnostics).[4]. After completion of the Alamar Blue assay, the microtiter plates were frozen for subsequent analysis of cell numbers using the Cyquant (Molecular Probes) assay for DNA content.

### Freezing

The freezing assay was performed using the same steps as for the cytotoxicity assay with the following modifications. Cell density for the freezing assay was $2 \times 10^4$ cells/well. After the final addition of CPA, the plate was cooled at −1°C/min to −80°C, then stored at −135°C (overnight) in a liquid nitrogen storage freezer. The next day the plate was removed from the freezer and allowed to equilibrate in a −20°C freezer for 30 minutes. Following equilibration, the plate was removed from the freezer and thawed rapidly in a 37°C water bath. During this period (~1 to 2 minutes), 0.5 mol/L mannitol media warmed to 37°C was added to the wells. The plate was then immediately removed from the water bath and put on ice for the remainder of the cryoprotectant dilution steps. The recov-

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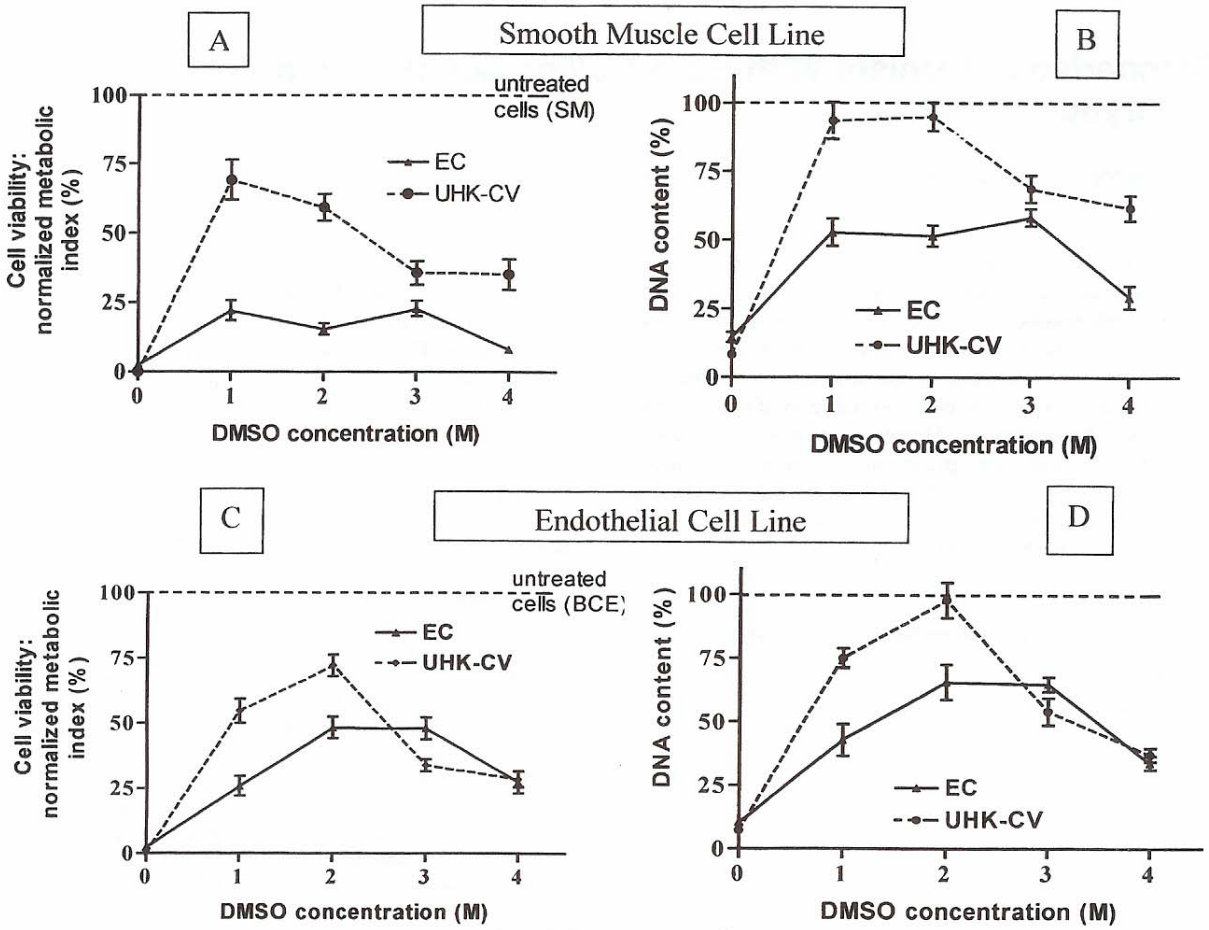


**Fig 1.** Cell survival. (Viability **(A,C)** and DNA content **(B,D)** after freezing and thawing with varying concentrations of DMSO in either Unisol-CV (UHK-CV) or Euro-Collins. Cells were frozen and thawed as adherent populations in microtiter plates. Data was normalized to untreated cells and represent the mean (±SEM) of 12 replicates.

ered cells in the plates were then warmed to 37°C for assessment of viability using Alamer Blue.

## RESULTS

In the absence of freezing there was no significant difference in the normalized viability of cells exposed to CPAs in Unisol-CV compared with Euro-Collins. In these studies, both cell types (smooth muscle and corneal endothelial cells) fully tolerated exposure to up to 2 to 3 mol/L CPA, but viability declined steadily after exposure to higher concentrations of CPA.

### Recovery After Freezing and Thawing

Figure 1A and C shows that cell survival was critically dependent upon the concentration of CPA, with optimum viability for both cell types being consistently obtained with 1 to 2 mol/L CPA in either vehicle solution. Using DMSO as the CPA, cell viability was consistently higher after treatment with Unisol compared with Euro-Collins and the highest recovery (~75%) after freezing and thawing was achieved with 1 to 2 mol/L DMSO. Interestingly, when PD was used as the CPA, optimum viability was significantly reduced compared with cryopreservation in DMSO (data not shown), and there was no significant difference between the two vehicle solutions. Cell recovery data curves (Fig 1B and D), derived from Cyquant DNA analysis, demonstrated a remarkably similar profile to the Alamar Blue metabolic indicator curves. This suggests that a physical loss of cells from the adherent layers used in these studies may account principally for the decreased survival observed under sub-optimal conditions of cryopreservation.

## CONCLUSIONS

1. This study illustrates that the selection of cryoprotectant, its concentration, and the nature of the vehicle solution are important variables that impact the outcome of cryopreservation. Moreover, these variables interact in an

unpredictable way such that each needs to be optimized for a given cell type.

2. The composition of the vehicle solution for CPAs is often overlooked as an important determinant of cell survival in cryopreservation. This study illustrates that the choice of carrier solution significantly impacts optimum survival and varies with the nature of the CPA and cell type.
3. High survival of cells frozen and thawed as an adherent population in microtiter plates can be achieved by optimizing the choice, concentration, and carrier solution of the cyroprotectant additives. This has important implications for practical applications of preservation in which cell adhesion to a substrate is critical such as in engineered tissues.

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