

Long-Term Preservation of Spheroids at $-85\text{ }^{\circ}\text{C}$ in a Liquid State

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Preserving spheroids for a long time is required in regenerative medicine and cancer research. Cryopreservation is essential to preserve spheroids, but the low temperature causes spheroids destruction by ice crystallization. Organic solvents are generally used as cryoprotective agents (CPAs) to inhibit ice crystallization during cryopreservation. However, most cryopreservation methods result in partly ice crystallization. We have proposed a new zwitterionic-based CPA whose freezing point is below $-85\text{ }^{\circ}\text{C}$.

The minimum concentration of organic solvents that maintained the liquid state at $-85\text{ }^{\circ}\text{C}$ was 60 wt%, but such solutions were highly toxic to spheroids. A zwitterionic liquid (10 wt%) that has low toxicity¹ and inhibiting ability for ice crystallization² (Fig. 1) was added. It also reduced the organic solvent concentration from 60 wt% to 50 wt%. However, such a concentration was still highly toxic to spheroids. We here mixed two organic solvents with a zwitterionic liquid to lose toxicity (Table 1) because mixing CPAs can reduce toxicity³. It was found that zwitterionic liquid/DMSO/EG/water (10/25/25/40, w/w/w/w) solution was the best combination for cryopreservation. This solution was less toxic than 50 wt% of a single organic solvent solutions and also inhibited ice crystallization. This combination also allowed the preservation of spheroids at $-85\text{ }^{\circ}\text{C}$ in a liquid state.

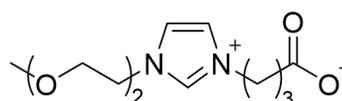


Fig.1 Zwitterionic liquid

Table 1 Minimum unfrozen concentration of single organic solvent solutions at $-85\text{ }^{\circ}\text{C}$

	Minimum unfrozen concentration organic solvent solutions at $-85\text{ }^{\circ}\text{C}$				
	DMSO	DMF	DMAc	EG	PG
-10 wt% OEzimC ₃ C	60%	70%	>80%	70%	60%
+10 wt% OEzimC ₃ C	50%	50%	50%	60%	60%

(CPA) DMSO: dimethyl sulfoxide EG: ethylene glycol
DMF: dimethyl formamide PG: propylene glycol
DMAc: dimethyl acetamide

Reference

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