Imperial College London

DMSO-free Cryopreservation of Cellular Products

Jie Ren, Dr Rongjun Chen*
Department of Chemical Engineering, Imperial College London

Introduction

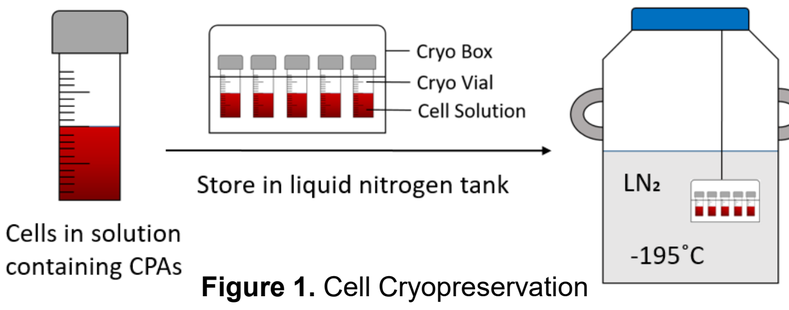

Figure 1. Cell Cryopreservation

Cell Cryopreservation, such as preservation of therapeutic cells including CAR-T cell, NK cell, stem cells is becoming increasingly crucial. Apart from that, cryopreservation of Red Blood Cells (RBCs) can greatly resolve the blood waste issue in blood banks worldwide. During Cryopreservation the ultralow temperature can lead to injury to cells, thus chemicals known as cryoprotectant (CPAs) which can protect cells have been explored. DMSO is the most widely used CPA yet its toxicity has greatly limited the application.

Trehalose, a disaccharide found in *tardigrade* which can bear extreme desiccation and cold, can work efficiently as a CPA when it is presented at both side of cells with limited cytotoxicity. Intracellular delivery methods such as microinjection, osmotic shock were developed yet failed due to insufficient delivery or safety concerns.

Novel polymer that is amphiphilic, pH responsive and biodegradable named PLP-NDA18 has been designed for intracellular sugar delivery. Sucrose itself as an alternative to DMSO as CPA for RBCs cryopreservation has also been investigated for the first time .

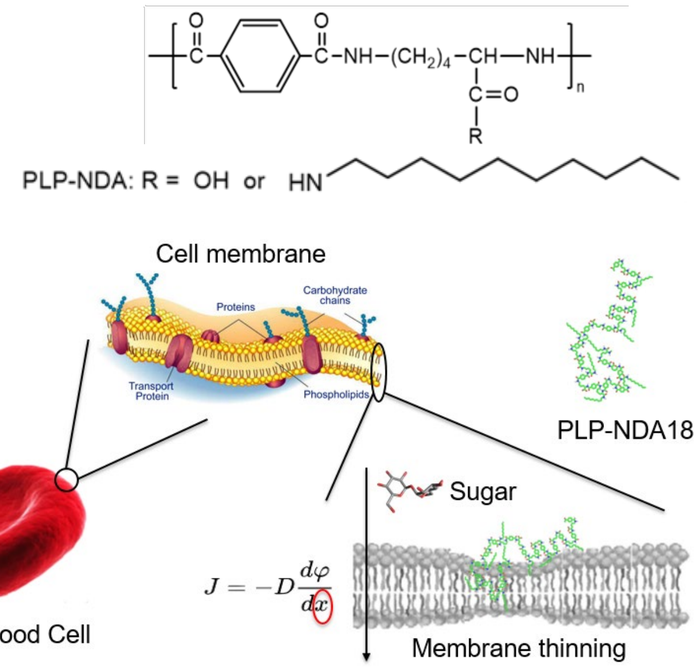

Figure 2. Chemical structure and mechanism of PLP-NDA18

Achievements

- ❖ Sucrose by itself for the first time has been proved to be an efficient CPA for RBCs cryopreservation resulting in a similar cryosurvival rate as that of trehalose;
- ❖ Cryosurvival rates of RBCs preserved by sugars were about 10% higher than that of DMSO, suggesting sugars can be alternatives to DMSO with lower toxicity;
- ❖ Post-thaw study showed a significant advantage of sugars as CPA: the cells can survive washing step while cells in DMSO group cannot.

Results

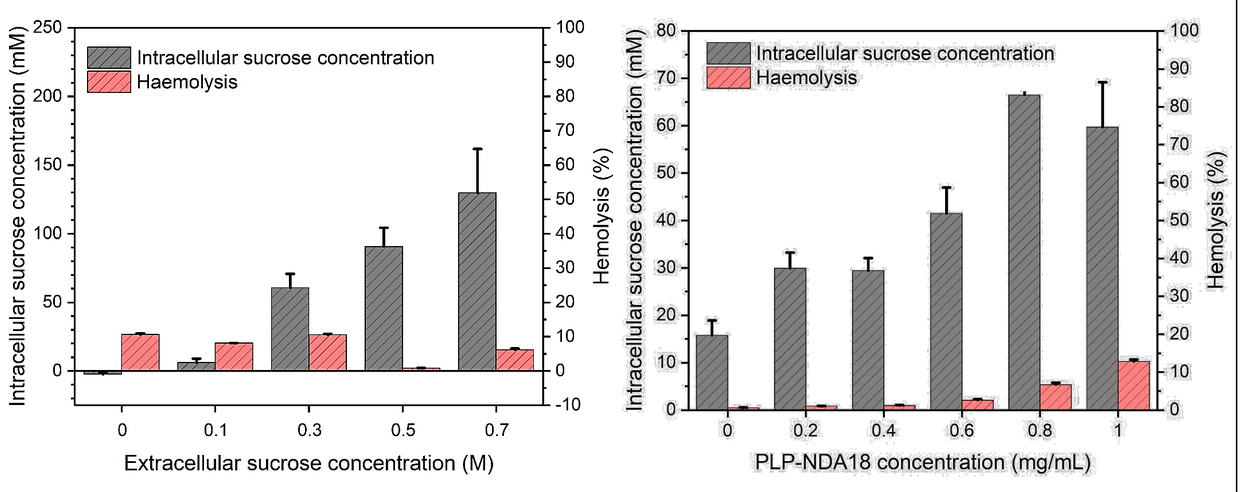

Figure 3. Loading optimisation: concentration of extracellular sucrose and PLP-NDA18

Increase of the extracellular sucrose concentration, PLP-NDA18 concentration and incubation time, decrease of pH can all end up with a higher intracellular concentration of sucrose. With the presence of PLP-NDA18 at an acidic pH, about 6 times more sucrose can be loaded into RBCs within 10 minutes compared with the condition that without PLP-NDA18.

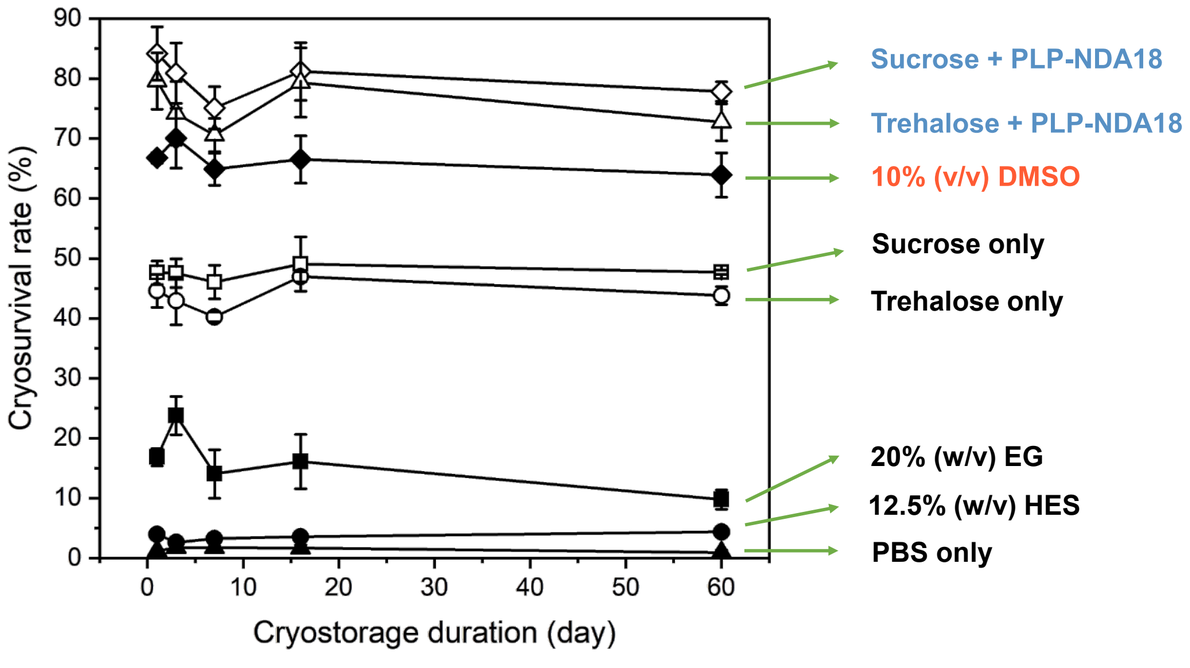

Figure 4. Cryosurvival rate of RBCs by different CPAs

After loaded with intracellular sugar, RBCs were cryopreserved with extracellular sugar at pH 7.4 for up to 60 days. Other CPAs such as ethylene glycol (EG), hydroxyethyl starch (HES) and sugars only in the extracellular environment were also investigated as comparisons. RBCs cryopreserved by sugars both inside and outside of cells ended up with the highest survival rates after thawing.

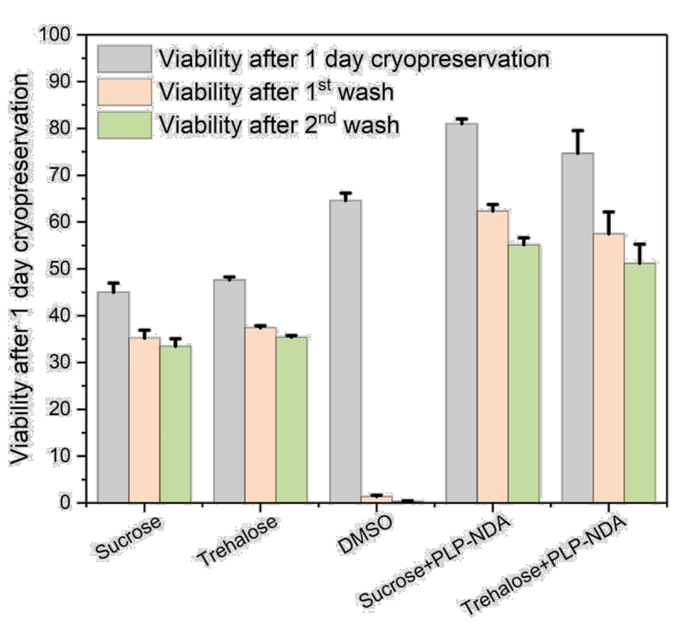

Figure 5. Viability of cryopreserved RBCs after wash

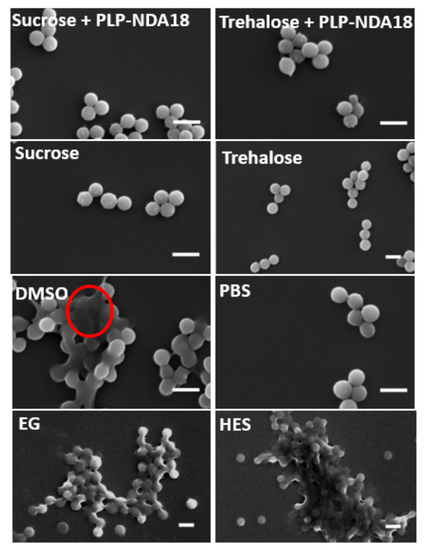

Figure 6. Scanning Electron Microscopy (SEM) of RBCs treated by different CPAs for 30mins

Thawed cells were washed by PBS and the viability was measured. Survival rate of RBCs in DMSO group dropped dramatically which may be due to the possible membrane fusion observed by SEM images.

Conclusion

1. Intracellular delivery of sucrose into RBCs was studied and parameters including concentrations of sucrose and PLP-NDA18, pH, incubation time were optimised for a sufficient loading.
2. Cryopreservation results showed that RBCs preserved by sugars both inside and outside of cells can end up with about 10% higher survival rate than RBCs cryopreserved by DMSO which is the routine method widely used currently in cell cryopreservation.
3. RBCs cryopreserved by DMSO were almost all dead after post-thaw wash while most cells in sugar groups stayed alive.

Acknowledgements

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References

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