Structure Optimization of Zwitterions for Cell Cryopreservation

Takeru Ishizaki,[a] Kojiro Ishibashi,[b] Eishu Hirata[b] and Kosuke Kuroda[a,*]

[a]*Faculty of Biological Science and Technology, Institute of Science and Engineering, Kanazawa University, Kanazawa, Japan*
[b]*Cancer Research Institute, Kanazawa University, Kanazawa, Japan*
**kkuroda@staff.kanazawa-u.ac.jp*

Cryopreservation is a technique that enables long-term preservation of cells at cryogenic temperatures. To prevent cell death caused by ice crystallization during freezing, cryoprotectants such as dimethyl sulfoxide (DMSO) are commonly used; however, DMSO exhibits cytotoxicity. We have reported zwitterions as novel cryoprotectants that exhibit lower toxicity than DMSO and strongly inhibit ice crystallization.[1] These zwitterions have been shown to preserve various cell lines and multicellular spheroids.[2,3] Nonetheless, certain cell lines remain challenging to preserve, indicating the need for further improvement. Therefore, this study aims to optimize zwitterion structure to improve cryoprotective effects.

Firstly, superfluous structures in zwitterionic cryoprotectants were investigated. The human lung cancer cell line PC9, which is easily cryopreserved using conventional zwitterions, was cryopreserved using 16 different zwitterions. As a result, increased hydrophobicity—due to long alkyl cation tails or spacers—reduced cell viability (**Fig. 1**), whereas cation and anion differences and oligoether side chains had no effect on viability.

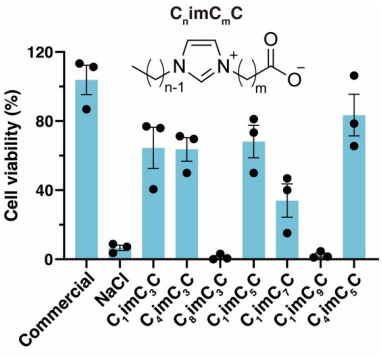

Fig. 1 Cell viability of PC9 after cryopreservation

Secondly, necessary structures to increase the cryoprotective effect of zwitterionic cryoprotectants were investigated. Human kidney cell line BOSC, which is difficult to preserve using zwitterions, was cryopreserved. Among the 16 zwitterions, two zwitterions showed high cell viability. To investigate the reason for this, the proportion of ice in each zwitterion solution after freezing was measured using a differential scanning calorimeter. The two zwitterions strongly inhibited ice crystallization, and zwitterions with carboxylate anions or oligoether cation tails strongly inhibited ice crystallization.

References

1. Kuroda, K. *; Hirata, E. *et al. Commun. Chem.* **2020**, *3,* 163.
2. Kato, Y.; Hirata, E.; Kuroda, K.* *et al. Commun. Chem*. **2021**, *4*, 151.
3. Ishizaki, T.; Hirata, E.; Kuroda, K.* *et al. Sci. Rep*. **2023**, *13*, 37.

Takeru Ishizaki
I am a Ph.D. student in the Kuroda group at Kanazawa University, where I have also completed my Bachelor's and Master's degrees. I have published two first-author papers in *Sci. Rep.* (2023) and *J. Phys. Chem. B* (2024), in addition to seven co-authored papers. I am currently supported by the JSPS Research Fellowship (DC2) and participate in the WISE Program for Nano-Precision Medicine. **E-mail**: takerutyler@stu.kanazawa-u.ac.jp