UNIVERSITY OF MINNESOTA
Mechanical Engineering

Ramen Spectroscope for Low Temperature Damage during Cell Freezing
Author: Qi Chen

Abstract

The objective of this project is to obtain more details by using confocal Ramen spectroscopy to see the effect of cryoprotectants such as Trehalose, glycerol, ectoine and their mixture on the protection of the cell during freezing and compare with the result from 1XPBS and DMSO. The jurkat cell was used as the sample cell and the test was performed starting from the $-6℃$ to $-50℃$ at a moderate cooling rate of $10℃/\mathrm{min}$. The mixture solution of 5% glecerol, 300mM trehalose and 0.02% ectoine was used as the protection solution. The CRM images were collected at the ultimate temperature ($-50℃$). Compared with the results achieved from the previous work (1×PBS and 10% DMSO solution). The cryoprotectants mixture presents a better protection effect during the cell freezing. The effect of cryoprotectants mixture at different cooling rates still needs to be studied for the future work.

Table of Contents

1 Introduction

Preserving a biological system during processing, transportation and storage is critical in many applications such as cell therapies and other medical uses. So much work has been done in the cell freezing. In the Previous work, the Ramen spectroscopy was utilized to observe the variation in cell at different cooling rates. Ramen spectroscopy allows us to optically section through the cell and obtain spectra at different focal planes. The spectra and its image can help us to determine the average ice crystal size and total volume of ice and variation of distribution of liquid water and organic material (micro-heterogeneity), which are markers of damage. Cell freezing in 1XPBS showed reduction in the size of cell during slow freezing and the corresponding lack of shrinkage observed at high cooling rates. In cells frozen in the presence of DMSO, spatial distribution of intracellular water was fairly uniform. As the cooling rate was increased, the distribution of intracellular water and organic material was left almost unchanged **[1]**. Besides the effect of the 1XPBS and DMSO, the effect of the cryoprotectant is quite of interest in our study. Therefore, the objective of this project is to obtain more details by using Ramen spectroscopy to see whether cryoprotectant such as Trehalose, glycerol, ectoine and their mixture can protect cell from the damage caused by freezing and compare with the result from 1XPBS and DMSO **[1]**. The jurkat cell will be tested at the moderate cooling rate of$10℃/\mathrm{min}$, with the protection of the cryoprotectant mixtures.

2 Materials and Methods

Equipment and Materials

One microliter of Jurkat cell suspension was placed on the surface of the temperature-controlled stage, covered with a piece of mica (1–3 mm in thickness), and sealed using Kapton tape (Dupont, Wilmington, DE), to prevent evaporation/sublimation.

CRM measurements were conducted using an Alpha 300R confocal Raman microscope (WITec, Ulm, Germany). The microscope was equipped with a UHTS200 spectrometer and a DV401 charge-coupled device detector (WITec). A 100×air objective was used for all measurements. An Ar-ion laser at a wavelength of 514.5 nm and 15 mW of power was used for excitation. A multistage Peltier system was constructed to achieve cooling rates in the range of 0.1–50℃/min down to a minimum temperature of$-50℃$. Liquid nitrogen was used to purge the sample stage to avoid condensation. Ice was nucleated by touching the sample with a liquid nitrogen-cooled needle at the predetermined nucleation temperature.

Method

10 mL Jurkat cell solution first was rotated by the centrifuge machine for 10 minutes for the refinement purpose. After the spinning, the solvent was extracted out and the solute (Jurkat cell) then mixed with the mixture solution of 5% glecerol, 300mM trehalose and 0.02% ectoine.

The Jurkat cell was cooled by the peltier system starting from the $-6℃$ to $-50℃$ at cooling rate of $10℃/\mathrm{min}$. Experiment was repeated in triple with 3–5 cells and the CRM images presented below are the representative.

3 Results and Discussion

Once the ramen spectra images of the cell freezing with the mixture of the three cryoprotectants were achieved, it is necessary to compare the image results with those with 1×PBS and 10% DMSO solution obtained from previous work.

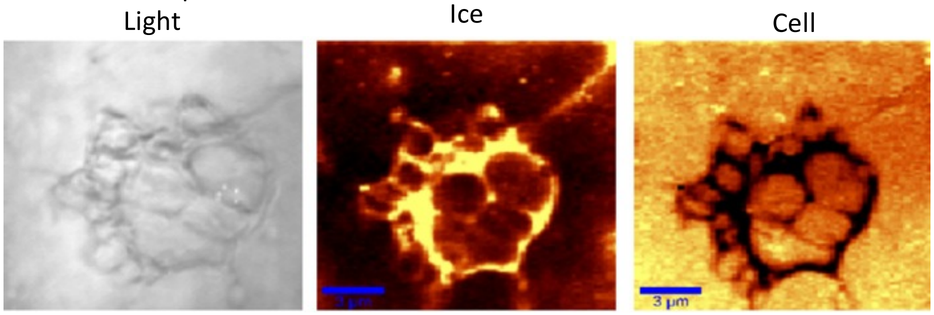

Figure 1: Jurkat cell in 1×PBS. Images at 100×magnification were collected at $T = -50°C$ after ice nucleation at $T_N = -2°C$ at $\frac{dT}{dt} = -10°C/min$

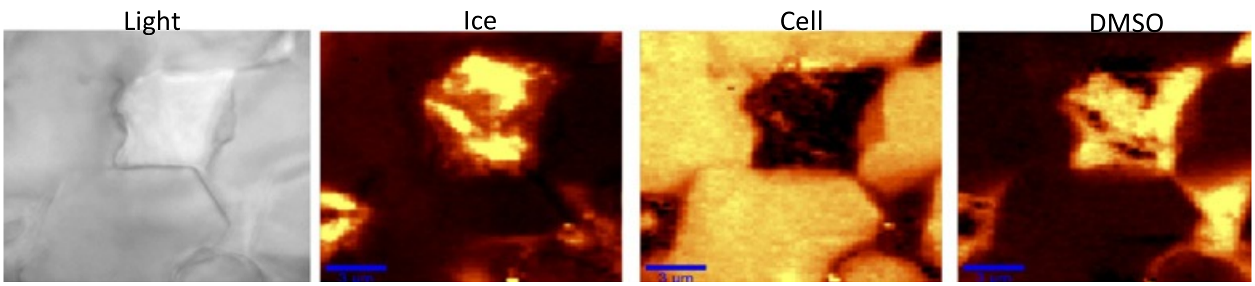

Figure 2: Jurkat cell in 10% DMSO. Images at 100×magnificationwere collected at $T = -50°C$ after ice nucleation at $T_N = -6°C$ at $\frac{dT}{dt} = -10°C/min$

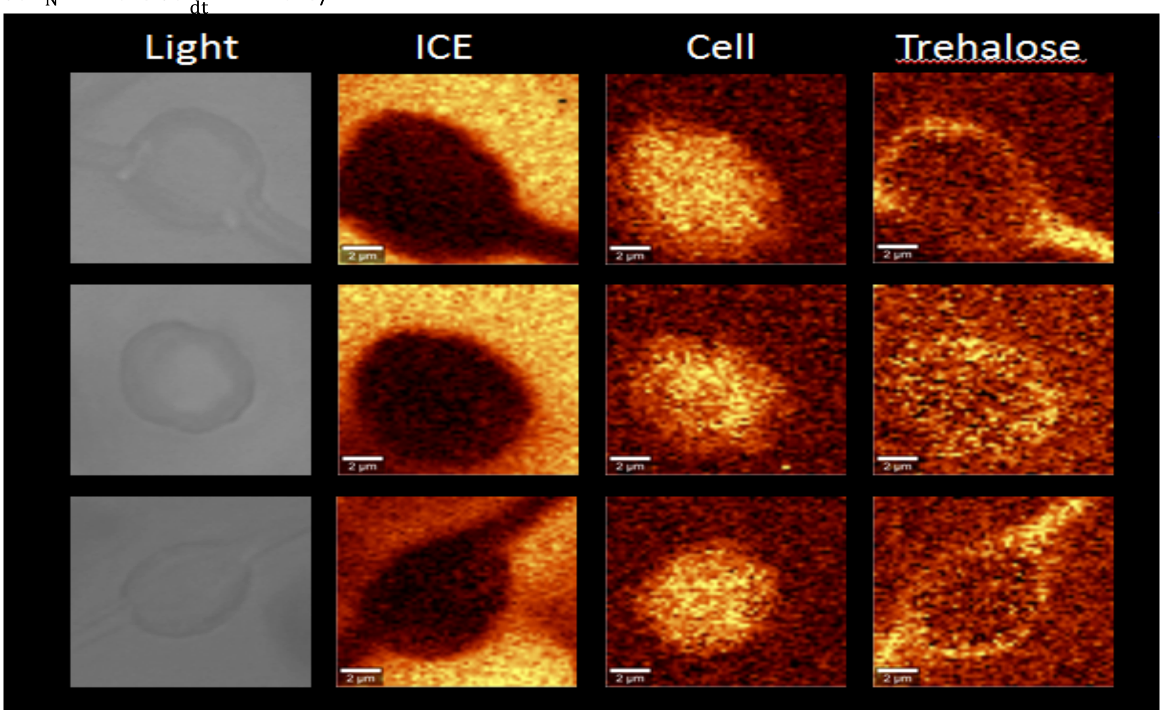

Figure 3: Jurkat cell in 5% glecerol, 300mM trehalose and 0.02% ectoine. Images at 100×magnificationwere collected at $\mathrm{T} = -50℃$ after ice nucleation at $\mathrm{T_N} = -6℃$ at $\frac{\mathrm{dT}}{\mathrm{dt}} = -10℃/\mathrm{min}$

Based on the light contrast images of Figure 3, the skeleton of the cell under cooling rate of $10℃/\mathrm{min}$ is almost kept in a complete shape. The ice and cell contrast images show that the ice does not break into the cell or forms inside, which means the cell does not undergo an obvious damage at the cooling rate of $10℃/\mathrm{min}$ with the protection of the mixture solution of glecerol, trehalose and ectoine.

However, in the trehalose contrast images, the second image shows that the trehalose penetrates into the cell, while the other two remain outside of the cell. This indicates that the cell have a slight freezing damage in the second trial.

Back to the image of 1×PBS and DMSO, for the 1×PBS in the Figure 2, all three contrast images (light, cell, ice) indicated that the cell had already broken down. For the 10% DMSO in the Figure 3, from the light contrast image, the cell was kept in a good shape, while from latter three images, they all showed that a few ice had formed inside the cell but the cell still get protected by the 10% DMSO.

4 Conclusions

With the facilitation of the confocal Ramen spectroscope, it is found that the mixture of cryoprotectants (glecerol, trehalose and ectoine) can better protect the cell during the freezing at a moderate cooling rate of $= -10℃/\mathrm{min}$, compared with the 10% DMSO and 1×PBS. The effect of the cryoprotectant mixtures at different cooling rates still needs to be studied for the future work.

References

1. Dong, Jinping., Malsam, Jason., Hubel, Allison., Bischof, C, John., and Aksan, Alptekin., October 2010. Spatial Distribution of the State of Water in Frozen Mammalian Cells. Biophysical Journal. 99: 2453–2459.