MICROSCOPIC STUDY OF CRYSTAL GROWTH IN CRYO-PRESERVATION SOLUTIONS

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Ice formation inside or outside cells during cryo-preservation has been proved to be the main cause for cryo-injury to cells. A high voltage DC electric field combined with a cryomicroscopic stage was used in this study. Dendritic ice crystals became asymmetric with the addition of electric field. DMSO and NaCl solutions were tested under different electric field strengths ranging from 83kV/m to 320kV/m. The shape change of ice crystals in the NaCl solution seemed more pronounced, because the NaCl solution was an ionic solution.

INTRODUCTION

It is generally believed that the survival of cells/or tissues is directly influenced by the extra- and intracellular ice formed during cryo-preservation processes[1-3].Consequently, eliminating the ice formation becomes the main topic of cryo-biologists.
In 1992, Hanyu et al. [4] introduced microwave irradiation at 2.45GHz during the cooling of samples. Compared with the non-irradiated samples, the irradiated samples had a deeper ice-free layer. The mechanism assumed was that the electromagnetic radiation broke the H_2O molecule chain and thereby disrupted crystal nucleation. A similar technique was used in 1997 by Jackson et al. [5] to test the behavior of cryo-preservation agent (CPA) solutions after the treatment by microwave irradiation. A bigger vitreous-like region was found in the pictures of the irradiated samples. The frequency of microwave used in the above two experiments was the same as the frequency of a domestic microwave oven that could agitate water and might kill the cells. A DC electric field was therefore used to replace the microwave irradiation in this study

MATERIALS AND METHODS

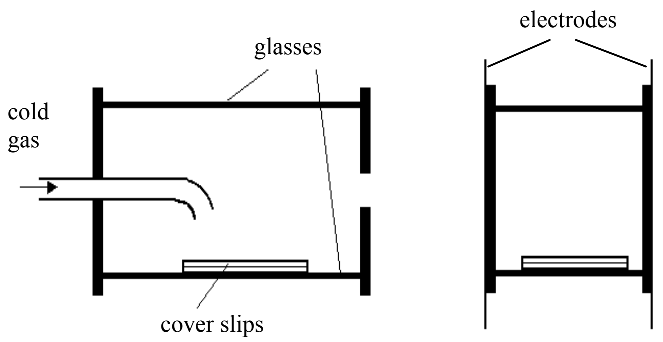

Fig.1 Test sector

A special apparatus was built to produce a DC field working with a cryomicroscopic system. Low temperature nitrogen gas was used to cool the samples. The gas from a high-pressure vessel passed through a relief valve and a flow meter and then was cooled by a plate-fin heat exchanger which was immersed in a liquid nitrogen (LN_2) Dewar. The lowest temperature that the gas could reach was 160 degrees in our experiments. The relief valve controlled the gas flux and, therefore, the sample's cooling rate was controlled too.
The samples used in this study were 5%(w/w) DMSO and 5%(w/w) NaCl aqueous solutions. The samples were held between two microscopic glass coverslips (Fig.1). One pair of electrodes was put in parallel at each end of the test section. The electrodes were made of copper and their surfaces were carefully polished to avoid possible sparks occuring while high voltage was applied. Two pairs

of thermocouples(copper-contantain) were placed on the surface of upper coverslip to measure the cooling rates. A unique temperature distribution was found by measuring the temperatures at different places on the coverslip surface.

RESULTS AND DISCUSSION

Freezing of DMSO solution

The continuous images of heterogeneous ice growth without an electric field are shown in Fig.2. A very tiny piece of ice was used as the embryo in the experiments, as seen in the first picture. The round embryo turned to be a hexagonal crystal in the second picture and the gradual symmetric hexagonal dendritic ice crystal growth is observed in Figs. 2(3) ,2(4) and 2(5).

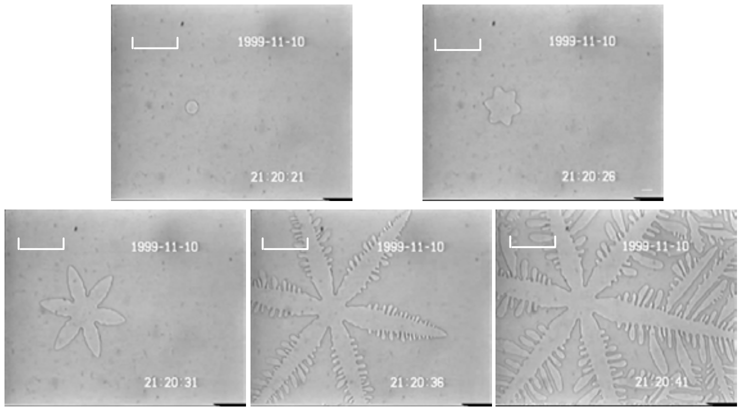

Fig.2 Ice growth of DMSO solution without electric field

Fig.3 demonstrates the course of ice crystal growth in the DMSO solution with an electric field. The direction of the electric field was vertically downward. The cooling rate was same as that of Fig. 2 and the ice crystal also grew from a very tiny embryo. Figs.3(3), 3(4) and 3(5) show that two main branches parallel to the electric field grew faster than the other branches.

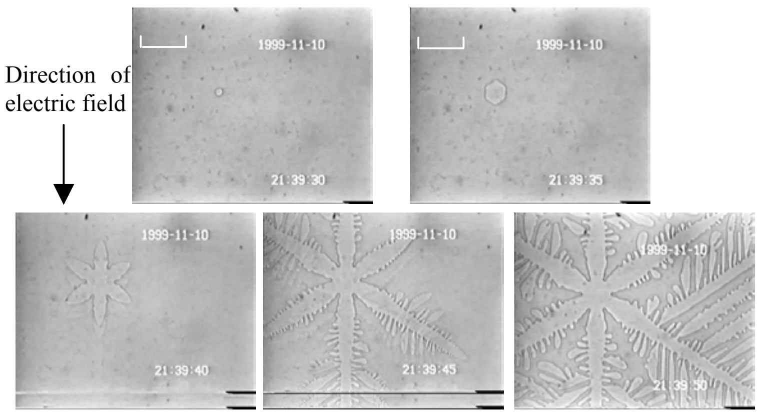

Fig.3 Ice growth of 5%DMSO solution under 250 (kV/m)

The effects of electric field on ice crystal growth had been numerically dicussed by Svishchev and Kusalik[6-7]. They announced that the strength of an electric field able to change the ice lattice from normal ice(I_h) to cubic ice (I_c)should be at least 10^5kV/m. However, the strength of electric field used in our experiments was only 1/400 of 10^5kV/m. Thus, the morphological change of the ice in this study was not caused by the ice lattice change. Without the electric field, the crystal growth process could be considered as a process whereby the water molecules are added one by one to the crystal lattice. This 'adding' process has normally the same probability in all directions, and leads to the formation of symmetric ice cyrstal (e.g. Fig. 2). However, when a high voltage field is applied, the electric field may cause different molecules in the DMSO solution to exhibit different behaviors. The polar water molecules/clusters may be torqued and rearranged under the action of electric field and forced to joining the lattice in a special orientation and position. Hence, different growth rates occurr in different directions and the ice crystal becomes asymmetric.

Under the action of an electric field, the water molecules may rearrange and line up end to end in the direction of the electric field. In viewing the crystal structure, this well-ordered water molecules/clusters seems like crystal or quasi-crystallines. In this case the water molecules/clusters possess an ideal situation for rapid crystal growth. That may be the reason why the main branches, which are parallel to the direction of the electric field, grow faster than the other branches (Fig.3).

Freezing of NaCl solution

The ice crystal growth in 5% NaCl solution under the electric field strength of 320kv/m at relatively slow cooling rate is shown in Fig.4. The time interval between each pictures was about 20 seconds. The asymmertric crystal growth, more so than those shown in Fig.3, can be observed in Fig.4(1) to Fig.4(5).

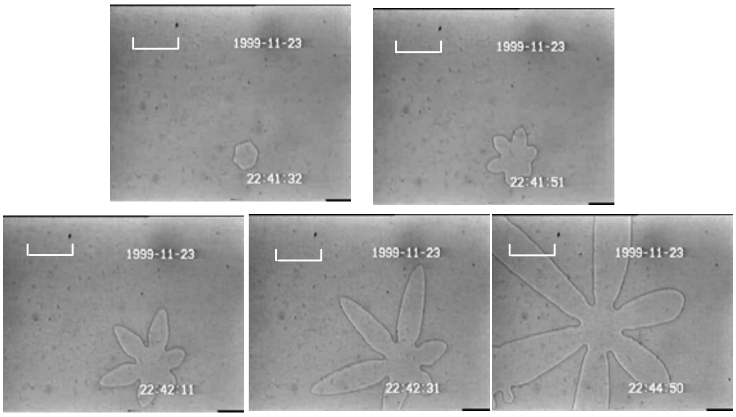

Fig. 4 Ice crystal growth of 5%NaCl solution under 320kV/m

The relative high electric field may be one reason for the ice morphological change. An alternative explanation is that the NaCl solution is an ionic solution, while the DMSO is not. The positive Na^+ and negetive Cl^- ions perform completely different movements when the electric field is applied. Ions could move and gather at the corresponding electrodes. This results in a non-uniform distribution of ions in the solution , and a different crystal growth rate could be expected in different regions of the solution. For the NaCl solution, the radius of Cl^- is much bigger than that of Na^+. That may also lead to a rather asymmetric ice crystal growth.

CONCLUSIONS

The morphology of ice crystal growth in 5%DMSO and 5%NaCl solutions in a DC electric field was studied. Different electric field strengths, from 83kV/m to 250kV/m, were added to DMSO solution. The asymmetric ice growth became more pronounced with the increase of the electric field strength,. At the field strength of 250kV/m, two main branches parallel to the field direction grew faster than others. Adding an electric field may force the water molecules/clusters to rearrange and line up and make the ice growth rate different in different direction. For freezing of NaCl solution, a more pronounced asymmetric ice crystal growth in the electric field of 320kV/m was noted. Ion separation under the effect of an electric field is regarded as the main reason for this observed phenomena.

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