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Effect of surface condition to temperature distribution in living tissue during cryopreservation

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Abstract. The temperature distribution of the simulated living tissue is measured for the improvement of the cooling rate during cryopreservation when the surface condition of the test sample is changed by covering the stainless steel mesh. Agar is used as a simulated living tissue and is filled inside the test sample. The variation of the transient temperature with mesh by the directly immersion in the liquid nitrogen is measured. The temperatures on the sample surface and the inside of the sample are measured by use of type T thermocouples. It is confirmed that on the sample surface there is the slightly temperature increase than that in the saturated liquid nitrogen at the atmospheric pressure. It is found by the comparison of the degree of superheat with or without the mesh that the surface temperature of the test sample with the mesh is lower than that without the mesh. On the other hand, the time series variations of the temperature located in the center of the sample does not change with or without the mesh. It is considered that the center of the sample used is too deep from the surface to respond to the boiling state on the sample surface.

1. Introduction

There are great hopes that the human ES / iPS cells are useful in the field of the regenerative medicine for the recovery of the lost capabilities by the illness and injury. The long term storage of these cells is done by the cryopreservation technique for the transportation or stock. However, there is a problem that the survival rate of these cells after freezing and thawing process is low [1]. The improvement of the cell viability is required in terms of the cooling rate and the cryoprotectant. The human ES / iPS cells have low freezing resistance and in the case of the low cooling rate, cells are damaged by the growth of the ice crystal. The growth of the ice crystals causes the dehydration, the deformation and the contraction of cells and the increase of the electrolytic concentration. On the other hand, in the case of the high cooling rate, the living cells freeze in the vitrification state and the damages of cells can be inhibited because of the suppression of the growth of ice crystals. Therefore, the high cooling rate is desired to improve cell viability in the cryopreservation. In fact, it is confirmed that the increase of the cooling rate changes the cell viability for the better from the experiment of the instantaneous freezing of the mouse skin-derived fibroblast cell line [2].

For the cooling method, the immersion in the cryogenic fluid is necessary to realize the high cooling rate. The liquid nitrogen is generally used for the cooling medium. However, the film boiling occurs on the surface of the cooling subject and consequently the heat transfer characteristics deteriorate. So, the film boiling condition occurred on the cooling subject must be controlled to promote the heat transfer characteristics.

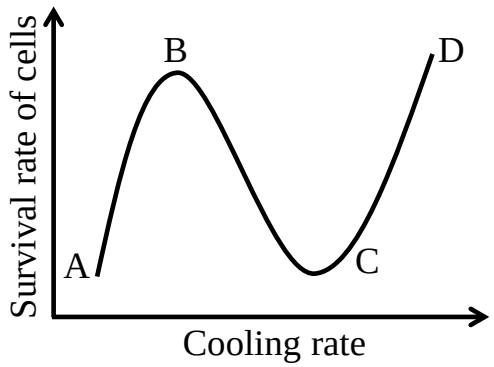

Figure 1. The qualitatively relationship between the survival rate of cells and the cooling rate [1].

In the present study, the film boiling occurred on the surface of the cooling subject during the immersion of the liquid nitrogen is tried to be controlled with the surface conditions. The surface conditions are changed by the stainless steel meshes. The agar is used as the simulated living tissues and is filled in the cryo-vial. Temperature distributions on the surface of the vial and in the center of the agar are measured. The effect of the surface condition of the cooling subject on the heat transfer characteristics is examined.

2. Cell viability and freezing pattern of ice crystals

The qualitatively relationship between the survival rate of cells and the cooling rate is shown in Figure 1 [3]. The vertical axis is the survival rate of cells and the horizontal axis is the cooling rate. It is seen that the survival rate increases in two areas. One is the area where the cooling rate is relatively high (designated in D). In this area, the vitrification occurs in the freezing pattern. The water inside and outside cells freeze instantaneously and solidify without the structure of ice crystals. In this case, the cells are hardly damaged since there is no growth of ice crystals. Another is the area where the cooling rate is relatively low (designated in B). In this area, the extracellular freezing occurs. First, water outside the cells freeze and become the ice crystals. And then the dehydration occurs by the difference in the osmotic pressure between inside and outside cells and the water inside cells moves to the outside and ice crystals grow outside cells consequently. Additionally, in the area C where the cell viability is low, the intracellular freezing occurs. In the intracellular freezing, a lot of sharp ice crystals occur inside the cells and cells damaged physically by sharp ice crystals. The values of the cooling rate corresponding to area B and C depend on the type of cells [4].

To achieve the high cell survival, it is necessary to inhibit the cell damage by the ice crystals and the high cooling rate is effective. And it is expected that the cell viability improves by increasing the cooling rate. In general, to achieve the high cooling rate corresponding to area D, cooling with liquid nitrogen in necessary. Hence, in the present study the increase of the cooling rate by the immersion in the liquid nitrogen is tried to be examined.

3. Experimental apparatus and procedure

In the present study, the temperature distributions in the agar as the simulated living tissue during the immersion in the liquid nitrogen are measured.

3.1. Test sample

The schematic illustration of the test sample is shown in Figure 2. The agar is used as the simulated living tissue. The concentration of the agar is 1.5 wt%. The living tissues in human body consist of about 70 % of water. So the water content of the simulated living tissue used in the present study follows

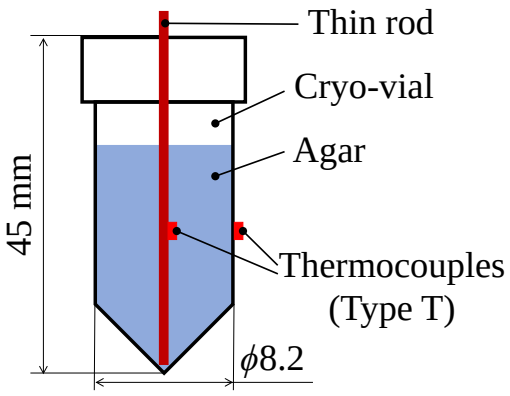

Figure 2. The schematic illustration of the test sample.

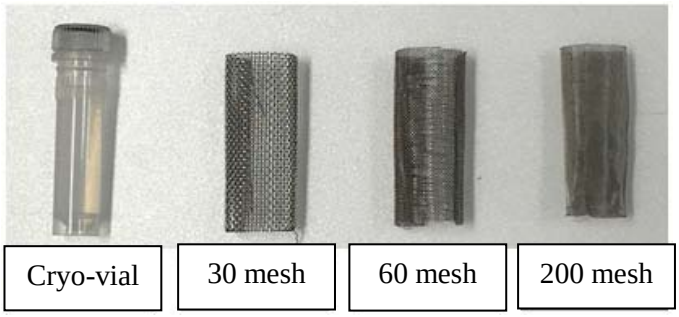

Figure 3. The picture of the cryo-vial and stainless steel meshes.

the actual human body. The agar is filled in a cryo-vial. The vial is the cylindrical shape and the size is ϕ8.2 x 45 mm. To change the surface condition, the stainless steel mesh covers the entire side surface of the sample. The picture of the cryo-vial and stainless steel meshes is shown in Figure 3. In the present study, three sizes of meshes are prepared: 30, 60 and 200 mesh/inch. The voids of each mesh are respectively about 500, 250 and 75 μm, and the diameters of wire of mesh are respectively 0.25, 0.14 and 0.05 mm.

For the temperature measurement, Type T thermocouples are installed on the surface of the sample and in the center of the sample. The positions of thermocouples are far from both of top and bottom of the vial. The size of the thermocouple installed on the surface is 0.127 mm in diameter and another thermocouple installed in the center of the sample is 0.2 mm in diameter. The thermocouple located in the center of the sample is 4 mm deep from the surface and supported by the thin rod in order to measure the same position under each condition. The nickel-chromium steel needle with a diameter of 0.8 mm is used as the thin rod. The thermocouple in the center is fixed on the rod covered with the polyimide tape for electrical insulation. The thin rod is in contact with the bottom of the vial and so there is a possibility of heat conduction through the rod. However, it is considered that the heat conduction through the rod does not influence the measurement of temperature in the center, since the rod is thin and the coefficient of thermal conductivity of nickel-chromium steel is relatively low compared with other metals.

3.2. Experimental procedure

The initial temperature of the test sample is 20 °C. The test sample is immersed in the saturated liquid nitrogen (-196 °C [= 77 K], 101 kPa) rapidly in the direction of the center axis of the sample. Temperatures are measured until the temperature of the center of the sample becomes steady state.

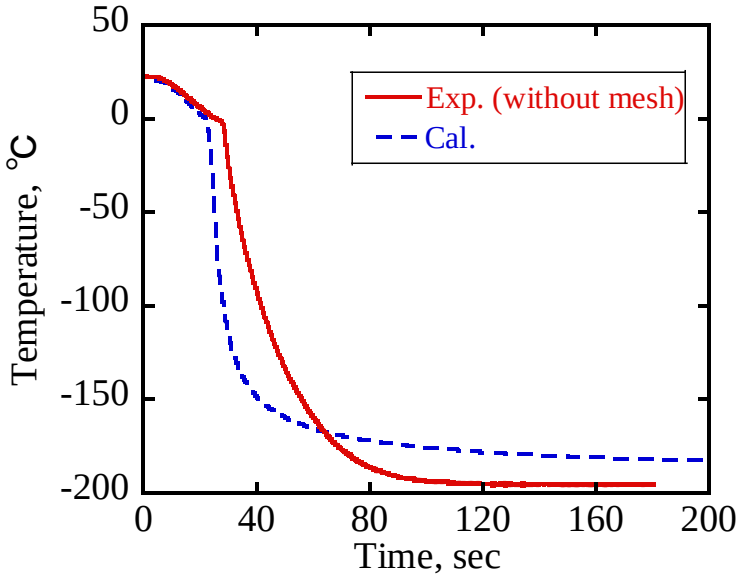

Figure 4. Comparison between the experimental results and calculated unsteady heat conduction.

4. Results and Discussions

4.1. Evaluation of experimental result by unsteady heat conduction

First, the experimental result is evaluated by the unsteady heat conduction in a semi-infinite body [5]. Time series variation of the temperature in the center of the test sample is shown in Figure 4. Shown in the solid line is the experimental result of the condition without mesh. It is seen that the temperature decreases gradually, but the temperature gradient changes in the temperature about 0 °C because of the phase transition from water to ice. This result can be compared with the calculated result by the unsteady heat conduction. The unsteady temperature variation T can be calculated by use of the error function

$$\frac{T-T_1}{T_0-T_1} = erf\left(\frac{x}{2\sqrt{at}}\right). \tag{1}$$

Here, T_0 is the initial temperature (= 20 °C), T_1 is the temperature of liquid nitrogen (= -196 °C), *erf* is the error function, x is the position from the sample surface, a is the thermal diffusivity and t is the time. Thermal diffusivity a is defined as

$$a = \frac{\lambda}{\rho c} \tag{2}$$

Here, λ is the coefficient of thermal conductivity, ρ is the density and c is the specific heat. These physical properties depend on the temperature and the phase changes from water to ice. So in the calculation, changes in the physical properties of both water and ice are taken into consideration [6]. The physical properties of living tissue are calculated from those of water as the physical properties of water are dominant for the living tissue. The broken line shown in Figure 4 is the calculated result. It is confirmed that up to about 60 seconds, the temperature of the experiment is higher than that of the calculation. It is considered that these differences are due to the thermal resistance of the cryo-vial.

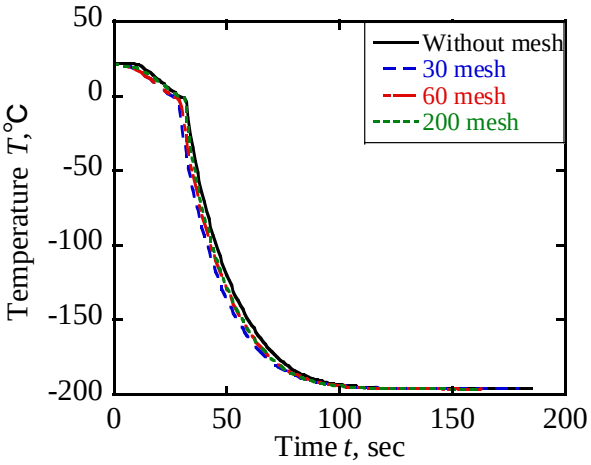

Figure 5(a). Time series variations of the temperature in the center of the sample.

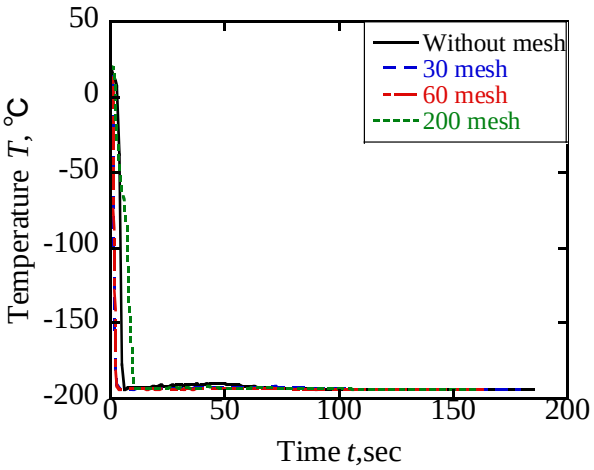

Figure 5(b). Time series variations of the temperature on the surface of the samples

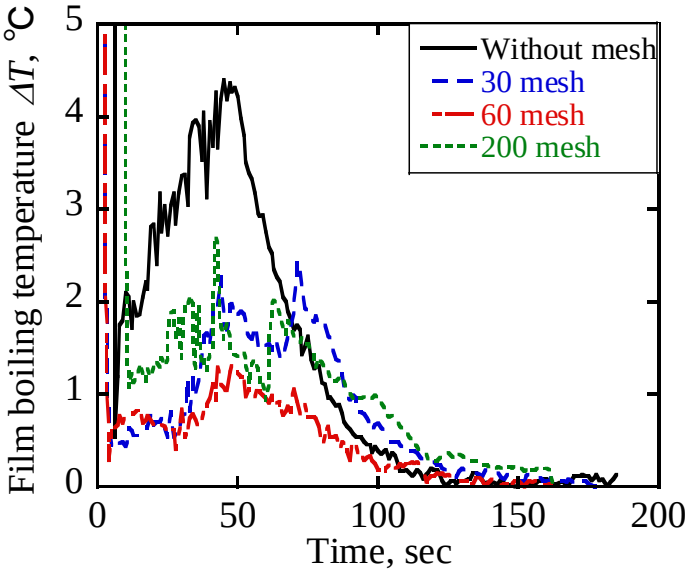

Figure 6. The detailed temperature variations on the surface of the sample.

4.2. Temperature variation of the test sample

4.2.1. Inside the sample. Time series variations of the temperature in the center of the sample are shown in Figure 5(a). The vertical axis is the temperature and the horizontal axis is time. 0 second corresponds to the onset of immersion in the liquid nitrogen. It is seen that the temperature in the center of the sample decreases gradually after onset of immersion and then temperature gradient increases because of the increase in the thermal conductivity by the phase transition from water to ice in the agar. The profile of any condition looks the same. It is considered that the depth of the center of the sample is 4 mm from the surface, so the heat conduction is dominant and the position of 4 mm from the surface is too deep to be affected by the surface condition.

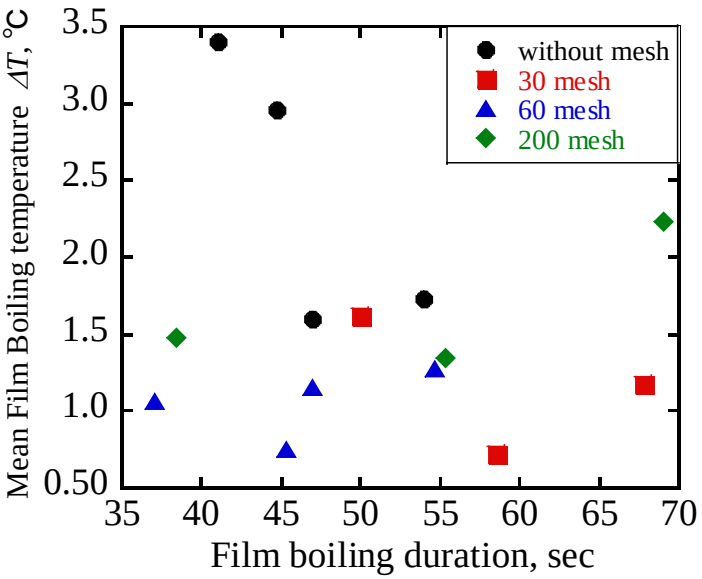

Figure 7. The relation between the mean film boiling temperature and the boiling duration

4.2.2. Sample surface. Time series variations of the temperature on the surface of the samples are shown in Figure 5(b). It is seen that in the temperature on the surface of the sample decreases rapidly just after onset of the immersion and temperature reach near the liquid nitrogen temperature. The detailed temperature variations on the surface of the sample are shown in Figure 6. The vertical axis is the temperature difference from liquid nitrogen temperature. The horizontal axis corresponds to Figure 5(b). It is found that temperature don't reach the liquid nitrogen temperature because of the generation of the film boiling. And the surface temperature during film boiling with the stainless steel mesh is lower than that without the mesh. While the film boiling occurs the temperature increases, and then the boiling state changes to the nucleate boiling, temperature decreases and reach the liquid nitrogen temperature.

4.3. The relation between film boiling temperature and film boiling duration

Next, the surface temperature during film boiling is investigated in detail. The relation between the mean film boiling temperature and the boiling duration is shown in Figure 7. The vertical axis is the mean film boiling temperature. All conditions correspond to the film boiling temperature in the range of about 0.50 °C ~ 3.5 °C and the boiling duration in the range of about 35 ~ 70 sec. It is found that the film boiling temperature without the mesh is higher than that with mesh. For the condition with the mesh, the boiling temperatures in the conditions with 30 mesh and 60 mesh are low, and in the condition with 60 mesh, the boiling durations are short. Therefore, it is considered that in the conditions with 30 mesh and 60 mesh the heat transfer during film boiling enhanced by the stainless steel mesh. And the conditions with 30 mesh or 60 mesh are suitable for the mesh size rather than that with 200 mesh. It could be estimated by the visual observations [7] that the thickness of the vapor layer during film boiling in liquid nitrogen was on the order of 0.1 mm. So in the case of 200 mesh, the thickness of the mesh might be too thin to influence the film boiling condition.

5. Summary

The temperature distribution with the surface condition during the cooling by the immersion of liquid nitrogen was investigated to improve the cell viability in cryopreservation. It is found that the increase of temperature on the sample surface during film boiling can be suppressed by covering with the stainless steel mesh.

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Acknowledgments

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