Application Note

CELLFLOAT

Data courtesy of Specially Appointed Associate Prof. Matsui, The University of Osaka.

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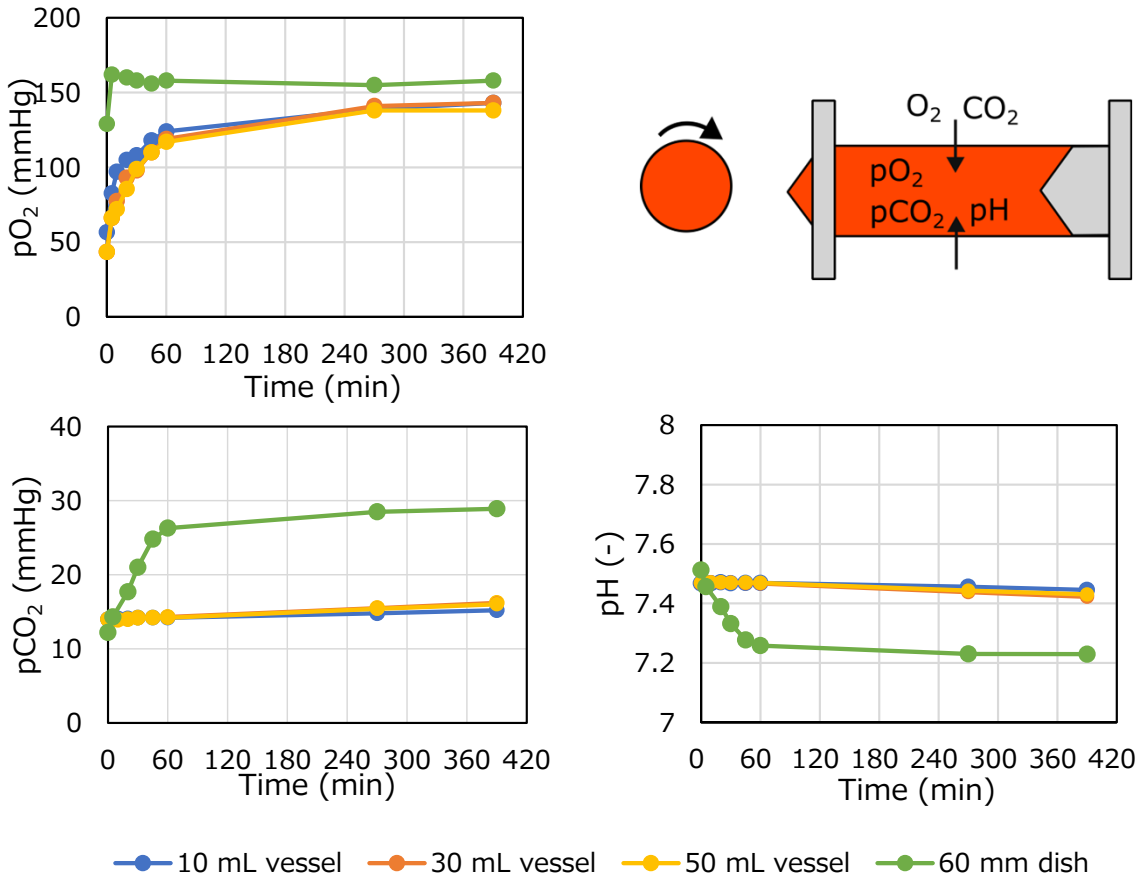
Gas Permeability Evaluation of Disposable Culture Vessels

Gas permeability, especially oxygen permeability, of closed culture vessels is very important for cell culture. To evaluate the gas permeability of the disposable culture vessels for CellPet 3D-iPS, we used nitrogen bubbling to remove dissolved gases from the culture medium, placed the medium in the culture vessels, and incubated them in a CO_2 incubator while rotating them, and measured the gas partial pressure using a medium analyzer. Furthermore, the gas partial pressure was measured under iPS cell culture conditions to evaluate whether sufficient oxygen permeated into the vessel and dissolved into the culture medium in relation to the oxygen consumption rate during cell culture.

Gas Permeability of Vessels

The culture medium AK02N (Ajinomoto) was nitrogen-bubbled to remove dissolved oxygen and carbon dioxide, and 10 mL, 30 mL, and 50 mL disposable culture vessels were filled culture medium to their respective maximum volumes. CellPet 3D-iPS were placed in a cell culture incubator in a 5% CO_2 environment, and the vessels were rotated at 40 rpm. Oxygen partial pressure pO_2, carbon dioxide partial pressure pCO_2, and hydrogen ion index pH were measured at each time point using a medium component analyzer, ViCell MetaFLEX (Beckman coulter). The pO_2 and pCO_2 partial pressures reached steady state quickly in the 60 mm dish, whereas the pO_2 reached steady state in about 5 hours in the disposable culture vessel at any volume. pCO_2 almost reached steady state in 60 min in the 60 mm dish, whereas pCO_2 increased very slowly in the culture vessels, and pH change was also slow.

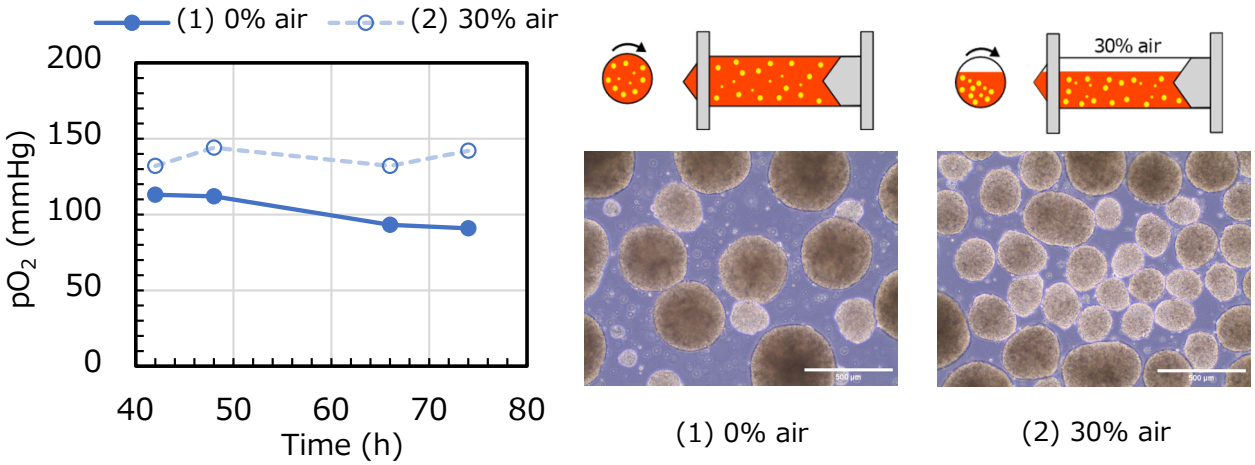


Medium gas partial pressure and pH in disposable culture vessels

Application Note

Gas partial pressure in culture vessels during iPS spheroid rotary floating culture

We evaluated the pO_2 of iPS cells (253G1) rotary floating cultured in disposable culture vessels for 3 days. To investigate the difference in the oxygen uptake into the culture medium, two culture conditions were used: (1) the vessel was completely filled with culture medium, and (2) the vessel contained 30% air phase. In the 0% air phase, pO_2 decreased with time. In contrast, pO_2 was maintained at approximately 140 mmHg in the 30% air phase. After 3 days of incubation, cell proliferation rates were (1) 240% and (2) 290%, respectively (initial seeding density 1 x 10^5 cells/mL). The 30% air phase condition is assumed to increase the rate of oxygen consumption by the cells because of the higher cell density. However, the oxygen partial pressure was maintained even when the oxygen consumption rate increased with cell proliferation because the oxygen was efficiently dissolved into the medium at the gas-liquid interface in the vessel. Although oxygen permeated into the medium through the vessel wall even under 0% air phase conditions as the gas permeability of the vessel was confirmed in the previous evaluation, pO_2 gradually decreased due to the higher oxygen consumption rate of the cells. The observed changes in the flow field in the vessel with and without gas phase also suggest that the growth rate of iPS cells and the particle size of spheroids are also affected.



Oxygen gas partial pressure in the medium of disposable culture vessels during iPS spheroid culture process

DISCUSSION

The gas permeability of disposable culture vessels for CellPet 3D-iPS was evaluated. The culture vessels were filled with culture medium from which dissolved gases were removed, and the time variation of the gas partial pressure was evaluated, and it was confirmed that the oxygen gas reached a steady state in about 5 hours. Additionally, the permeability of the oxygen dioxide gas was low, and the pH change was slow. The oxygen partial pressure in the medium during the rotary floating culture of iPS spheroids was evaluated, and it was found that the oxygen partial pressure could be maintained by adding an air phase in the vessel. Although it depends on the cell density, it is suggested that the oxygen partial pressure can be maintained by including a gas phase in the vessel when culturing cells with a high oxygen demand.

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