

Evaluation of respiration activity of bovine milk by SECM for diagnosis of mastitis

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[Introduction] Currently, the somatic cell count (SCC) is widely used as a mastitis diagnosis method. It is diagnosed as mastitis when the number of somatic cells in bovine milk is 1.0×10^7 cells/mL or more. This indicates an advanced state of inflammation, which makes early diagnosis difficult. In this study, we tried to evaluate the respiration activity of somatic cells in bovine milk using Scanning Electrochemical Microscopy (SECM)¹. **[Methods]** We centrifuged the bovine milk and removed the cell suspension (Fig.1A). We inserted this cell suspension into an inverted conical well (both the radius and height are 2 mm). The respiration activity was measured by scanning a Pt microelectrode in the Z direction between 20 μm and 1020 μm from the cell suspension while measuring the oxygen reduction current (Fig.1B). **[Results and Discussion]** Fig.2 shows the measurement results of 6.0×10^6 cells/mL and 6.0×10^5 cells/mL. We use a dissolved oxygen concentration of 209 μM in PBS at 37 °C, and an oxygen reduction current value based on this at -3.0 nA. We calculated the oxygen consumption of the cells from the difference in the current value between O and N1 and between O and N2. A suspension of 6.0×10^6 cells/mL was determined to be approximately 7 μM , and a suspension of 6.0×10^5 cells/mL was determined to be approximately 1 μM . These graphs suggest that there is a correlation between the cell number and respiration activity. We are examining the creation of a calibration curve for the number of somatic cells in bovine milk by evaluation of the respiration activity. **[Reference]** (1) H. Kikuchi, A. Prasad, R. Matsuoka, S. Aoyagi, T. Matsue, S. Kasai: *Frontiers in Physiology* 7, 25, 1-6 (2016).

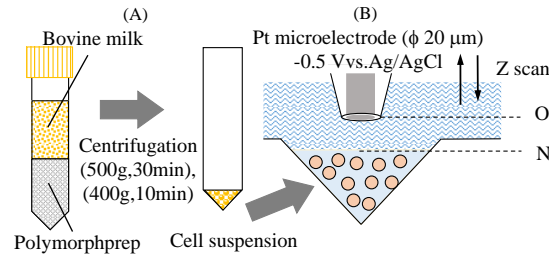


Fig.1 Schematic representation of setup for evaluation of respiration activity
 (A) The Polymorphprep was added to a centrifuge tube, bovine milk was added from above, and centrifuged.
 (B) The Pt electrode was scanned in the Z direction at the center of the well with the cell suspension at room temperature in 10 mL phosphate-buffered saline (PBS) buffer containing 11.4 mM glucose. The scanning distance, scanning speed and sampling time are 1000 μm , 50 $\mu\text{m}/\text{s}$ and 100 msec, respectively.

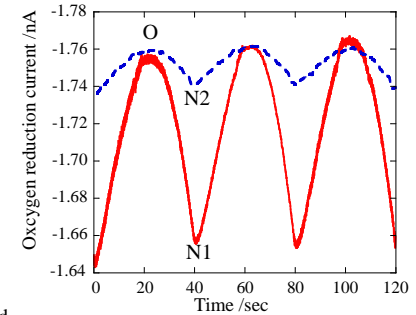


Fig.2 Evaluation of respiration activity comparison of the magnitude of oxygen reduction current using SECM in the presence of milk
 The solid line shows the respiratory activity with the cell number of 6.0×10^6 cells/mL.
 The dashed line shows the respiratory activity with the cell number of 6.0×10^5 cells/mL.