

## Evaluation of respiratory burst of bovine milk by SECM for diagnosis of mastitis

(Faculty of Engineering Tohoku Institute of Technology<sup>1</sup> · Graduate Department of Electronics Tohoku Institute of Technology<sup>2</sup> · Morinokuma Animal Hospital<sup>3</sup>)

○Haruka Takanashi<sup>1</sup>, Ryoma Kumagai<sup>2</sup>, Saya Saito<sup>1</sup>, Yutaro Takahashi<sup>2</sup>, Yusaku Sato<sup>2</sup>, Masashi Kumagai<sup>3</sup>, Shigenobu Kasai<sup>1,2</sup>

**[Introduction]** The somatic cell count (SCC) is widely used as a mastitis diagnosis method. However, this method cannot determine the cell type. Therefore, it is difficult to know whether the immune cells increase during the early stage of mastitis, thus early diagnosis is difficult. We have electrochemically evaluated the respiratory burst (Fig.1A) when neutrophils and monocytes are triggered by phorbol 12-myristate 13-acetate (PMA)<sup>1)</sup>. In this study, we describe the results of examining the respiratory burst evaluation of neutrophils and monocytes contained in bovine milk using a scanning electrochemical microscopy (SECM). **[Methods]** As shown in Fig.1, we added 2 μL of bovine milk to an inverted conical well (the radius and height are both 2 mm) and measured the oxygen reduction current using a Pt microelectrode. In addition, we injected PMA to induce a respiratory burst and continued to perform the amperometry. **[Results and Discussion]** Fig.1 shows the measured amperogram. Immediately after inserting the PMA, there is a rapid change in the oxygen reduction current, and it is considered that a respiratory burst was obtained from normal breathing. This shows the same shape for the phenomenon obtained in the cell line of human monocytes<sup>1)</sup>. After about 16 minutes, it returned to a normal respiration activity. In the future, we plan to compare mastitis bovines and healthy bovines. **[Reference]** (1) H. Kikuchi, A. Prasad, R. Matsuoka, S. Aoyagi, T. Matsue, S. Kasai: *Frontiers in Physiology* 7, 25, 1-6 (2016)

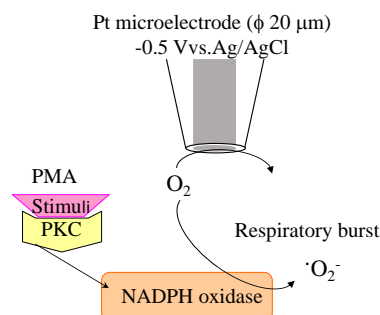


Fig.1 A respiratory burst pathway near the cell membrane of neutrophils or monocytes and its electrochemical measurement conceptual diagram. The oxygen reduction current was measured by applying -0.5 V vs. Ag/AgCl to the Pt microelectrode at room temperature in 10 mL phosphate-buffered saline (PBS) buffer containing 11.4 mM glucose. 10 μL of 100 μM PMA was added to induce a respiratory burst.

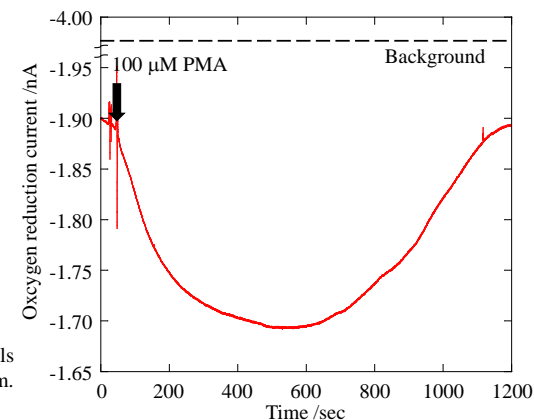


Fig.2 Measurement of oxygen consumption of immune cells in bovine milk. 10 μL of 100 μM PMA was added at the arrow.