学 位 論 文 内 容 の 要 旨

博士の専攻分野の名称　博士（理学）　氏名　Luo Dan

学 位 論 文 題 名

Study of Extracellular Electron Transfer in Bio-corrosive Bacteria for Electrochemical Sensor Application

（電気化学センサー応用に向けた腐食性細菌の細胞外電子移動に関する研究）

Microorganisms hold a pivotal ecological position on Earth, exerting significant influence on both human physiology and the surrounding environment. Most microorganisms in anoxic environment lacks the capacity to utilize oxygen as an electron acceptor and are thus capable of deriving energy through fermentation or electron transfer to alternative inorganic terminal electron acceptors. The microorganisms possess the ability to transfer electrons from the intracellular to the extracellular environment, termed electrochemically active microorganisms (EAMs), and this process, known as extracellular electron transfer, holds considerable importance. Biocorrosion caused by microbial biofilm occur in many places, not only on metal surface (sewer pipes) but also dental material, known as microbiologically influenced corrosion (MIC). The biosensor of electrogenic bio-corrosive bacteria serves a straightforward and readily implementable design, rendering it accessible for practical application to diagnose environmental biocorrosion compared with other biosensors. Furthermore, this biosensor possesses the unique capability to assess cell activity and evaluate drug efficacy, surpassing the functionalities offered by alternative biosensor systems.

In **chapter 1**, an introduction and comprehensive review of electrogenic bio-corrosive pathogens found in iron and dental bio-corrosive environment was provided. The significance and imperative nature of developing biosensors targeting these bio-corrosive pathogens were emphasized. Additionally, the mechanisms underlying extracellular electron transfer (EET) and uptake (EEU) were summarized, along with the discussion of their conventional applications, including biocorrosion. The chapter highlighted the potential for developing electrochemical biosensors based on EET or EEU capability of representative bio-corrosive bacteria. The author mainly researched on the extracellular electron transport process of two typical bio-corrosive species in different environment for the development of electrochemical biosensors. One is iron-corrosive bacteria *Desulfovibrio ferrophilus* IS5 (IS5), a model EEU bacteria for microbiologically influenced iron corrosion (MIC), and the other one is an EET-capable bacteria with undefined EET mechanism, *Porphyromonas gingivalis* (*P. gingivalis*), an important periodontal pathogen causing dental corrosion. In this thesis, the author examined the electrical interaction between electrode and electrogenic bio-corrosive bacteria (IS5 and *P. gingivalis*) to enhance the biosensor sensitivity and selectivity. In details, the effect of electrode surface property, metabolic pathway and shuttles on electron transfer rate was studied. The author even investigated human saliva samples for periodontitis biosensor development.

In **chapter 2**, the author examined the impact of electrode wettability on the electron transport mechanism at the interfaces between cells and electrodes as well as the sensitivity of EEU-based electrochemical biosensor of IS5. The impact of the physical attributes of the substrate on the electron transport capability of IS5 holds valuable implications for the advancement of electrode materials in IS5 biosensors, as well as for the prevention of iron corrosion. First, the wettability of indium-tin-doped oxide (ITO) electrodes was modified by dip-coating them with hydrophilic NH_2- or SH- ligands or hydrophobic CH_3- ligands. It is reported that EET capable strain *Shewanalla loihica* PV-4 generated higher current on more hydrophilic electrode which provided more reduced *C*-type cytochromes located on the outer membrane (OMCs). Similar with *Shewanalla loihica* PV-4, IS5 showed current generation coupled with sulfate reduction increased by as much as nine-fold with increasing electrode hydrophilicity, and displayed a positive linear

correlation with the number of cells attached to the electrodes. Hence, IS5-mediated EEU most likely requires direct cell attachment to the electrode surfaces. Differential pulse voltammetry showed that electrode wettability altered the peak potential and intensity of the IS5 reductive signal possibly because of direct interactions between OMCs in IS5 and the electrode surfaces. These results support the hypothesis that IS5 utilizes an OMC-mediated direct EEU mechanism, which has implications for the mechanism underlying corrosion, EEU-based electrochemical biosensor development and anti-biocorrosive material.

In **chapter 3-4**, the author further studied the effect of substrate metabolism and redox mediators on electron transfer process of bio-corrosive bacteria and designed a highly sensitive and specific EET-based electrochemical biosensor of *P. gingivalis* for the diagnosis of dental biocorrosion as well as periodontitis. Recently, some harmful bacteria in dental environment including *P. gingivalis,* which is an important biomarker of periodontitis, were found to be capable of EET with sugars oxidation accompanied by the production of organic acids causing dental biocorrosion. However, the EET mechanism of *P. gingivalis* is unclear. In **chapter 3**, the author first utilized electrodes with different wettability, which affected OMCs redox state, to study the redox protein on outer-membrane of *P. gingivalis* for electron transfer from intracellular to extracellular. Besides, based on the specific amino acid metabolism pathway of *P. gingivalis*, the effect of different amino acids, peptides and sugars was examined on current production of *P. gingivalis*. It was found that with histidine, *P. gingivalis* generated the highest current compared with other substrates. Notable, other known oral EAMs including *Aggregatibacter actinomycetemcomitans*, *Streptococcus mutans*, *Corynebacterium matruchotii* and *Capnocytophaga ochracea* can hardly generate current except *P. gingivalis* generated high current with histidine. The potential metabolic models were simulated to compare the metabolic pathways among pathogens by using metabolic flux analysis such as NADH and ATP. The author further electrochemically analyzed human saliva collected from a healthy (without *P. gingivalis*) moderate-healthy (with low percentage of *P. gingivalis*) and un-healthy (with high percentage of *P. gingivalis*) volunteer using a three-electrode electrochemical system with three substrates, glucose, lactate and histidine. It is as expected that patient sample produced a significantly higher current only with histidine, while healthy sample generated similar current with all substrates. Furthermore, the generated currents exhibited a gradual decrease in unhealthy, moderate-healthy, and healthy sample, which displayed a consistent trend with the observed number of pathogenic microorganisms. These results strongly prove the possibility of EET-based electrochemical biosensor (EECB) for detection of *P. gingivalis* in human saliva for home care.

In **chapter 4**, the author studied the *P. gingivalis* current enhancement using various types of mediators for improving the EECB sensitivity and time response. Although the current production of *P. gingivalis* with histidine as the substrate is the highest, it was still a weak signal on screen-printed electrode for the real application. In addition, the response time of the system was considerably prolonged (6-9 hours). It is well-known that current generation is strongly affected by the different redox mediators. Therefore, a high-throughput system with 96-well screen-printed carbon electrode was used for screening various mediators to boost the current production of *P. gingivalis*. The 2-hydroxy-1,4-naphthoquinone (HNQ) showed the best current production, and therefore was chosen as the best candidate for developing and designing the *P. gingivalis* biosensor. Our developed *P. gingivalis* biosensor based on EECB shows sensitive detection at low number of cells (high current production for hundreds of times higher than without HNQ) and fast response with less than 30 min. Real monitoring of *P. gingivalis* has been carried out in real human saliva samples to confirm the on-site detection of *P. gingivalis*. The detection time was less than 5 min with high current production, leading to design fast response and sensitive *P. gingivalis* biosensor based on EECB in human saliva sample. Our designed biosensor shows a significant progress in sensing and time response as compared to other biosensors (hours to days).

In **chapter 5**, the author concluded all researches in this study and gave some prospects in the research field. Based on our findings, the author proposed several promising avenues for future research in this field. Specifically, it is crucial and imperative to further explore the intricacies of the EET mechanism and enhance the electron transfer rate in bio-corrosive bacteria using alternative methodologies. This endeavor is of utmost significance to facilitate the development of an electrochemical biosensor for biocorrosion, characterized by high sensitivity and rapid response, in diverse environmental conditions.