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EXPERIMENTAL STUDY

Use of microbial fuel cells to monitor current production in *Qi*-deficient liver cells

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Abstract

OBJECTIVE: To monitor current production in *Qi*-deficient liver cells, and to study how cellular proton leakage might affect electric current production.

METHODS: Cells were placed in an microbial fuel cells (MFC) anode and the electric current was measured. Mitochondrial-affecting chemicals, 2,4-dinitrophenol (DNP) and resveratrol (RVT), were used to induce proton leakage in cells and their effect on current production observed. MCF-7 breast cancer cells exhibited higher proton leakage relative to normal liver cells. A mouse model for *Qi*-deficiency was prepared according to the Methodology of Animal Experiment in Chinese medicine. The *Qi*-tonics Buzhongyiqi Tang (BZYQT), which is used to treat the *Qi*-deficiency condition, was applied to *Qi*-deficient liver cells to examine how current production was altered.

RESULTS: Adding either DNP or RVT to normal liver cells increased current production. MCF-7 cells that possessed high proton leakage were also found to produce higher currents than normal liver cells. Higher current production, lower cellular glucose content, and lower adenosine triphosphate (ATP) production rate were found in *Qi*-deficient liver cells, in which the use of DNP or RVT further increased current production. The use of BZYQT to treat *Qi*-deficient liver cells decreased current production, counteracted the action of DNP, and also improved cellular glucose content.

CONCLUSION: High electric current production was found in liver cells with high cellular proton leakage. Positive current responses to both mitochondria-affecting chemicals, DNP and RVT, appeared to indicate proper mitochondrial function. The high proton leakage detected in *Qi*-deficient liver cells might have caused high energy losses, which served to explain the observed lower cellular glucose content and ATP production rate than in normal cells. These results might also explain the exhibited syndromes of low energy and fatigue in *Qi*-deficient patients. Proton leakage, induced by DNP or the *Qi*-deficient condition, was possibly caused by unusual uncoupling of oxidative phosphorylation and appeared to be inhibited by treatment with BZYQT, such that decreased current pro-

duction was observed after BZYQT treatment.

Key words: *Qi* deficiency; Hepatocytes; Bioelectric energy sources; Electric current; Proton leakage; 2, 4-Dinitrophenol; Resveratrol; Oxidative phosphorylation

INTRODUCTION

A microbial fuel cell (MFC) is a device that utilizes the respiratory metabolism of bacteria to produce electricity from various organic substrates.[1] Catalytic oxidation of organic substrates by bacteria at an anode releases electrons that eventually reach the cathode *via* an external electrical circuit, thus generating an electric current. Most MFC studies have concentrated in the goal of improving power output. Some of these devices might be modified for applications in bioremediation, as in MFC-based technologies to remove aqueous nitrate.[2] However, there remains a lack of in-depth understanding of extracellular electron transport associated with MFC operations. Although the exact mechanism of electron transfer from cells to the anode is as yet unclear, it is quite clear that electrical energy is derived from substrate metabolism *via* the respiratory metabolism of bacterial cells.

Electric power from an MFC is limited by the problem of over-potentials that hinder electron flow from bacteria to the electrode and thus decreases the electric potential across the MFC. These factors also include the electrical resistances of the electrodes, membranes, and electrolytes. Suboptimal contacts or low electrolyte conductivity increase the internal resistance of an MFC and decrease power generation.[2] To keep variations in internal resistance to a minimum, the same operating procedure must be strictly followed in each experiment. Any change incurrent measurement should then reflect cellular biochemical changes.

During respiratory metabolism in organisms, protons and electrons are produced in the mitochondrial electron transport chain (ETC). Hydrogen ions are pumped from the matrix, across the inner membrane, and into the intermembrane space, which creates a high inner membrane potential. This high proton-motive force drives protons across the inner membrane back into the matrix via adenosine triphosphate (ATP) synthase, which converts adenosine diphosphate to ATP.[3] However, the fact is that not all hydrogen ions flow through ATP synthase from the inner membrane, as some of them escape in an earlier ETC step. This uncoupling phenomenon between electron transfer and ATP synthesis is termed proton leakage, which leads to reduced ATP production.[4] Some energy that are lost because of proton leakage is released as heat to the surroundings and accounts for 20%-30% of heat generation by basal metabolism.[5]

As the degree of cellular proton leakage relates the loss of protons and electrons from the cell, it is reasonable to hypothesize that the degree of proton leakage might improve extracellular electron transport and reduce activation losses for electricity production.[2] To confirm the involvement of proton leakage during ETC in mitochondria with electric current production by cells, different cells with different degrees of proton leakage conditions, including normal liver cells, and high proton leaking cancer cells can be used in experiments to study the relationship between proton leakage and electrical measurements in an MFC. Proton leakage through various mechanisms can be induced by select chemicals, including resveratrol (RVT) and 2,4-dinitrophenol (DNP). RVT increases the mitochondrial membrane potential by inhibiting ATP synthase activity in a dose dependent manner,[6] leading to increases in cellular proton leakage. DNP increases proton leakage by enhancing proton conductivity across the mitochondrial inner membrane[7] and mild uncoupling of oxidative phosphorylation.[8]

As low energy generation and metabolism[9] have been described in *Qi*-deficiency of Traditional Chinese Medicine (TCM), it would be of great interest to examine how the low energy status of such cells affects current generation in an MFC. *Qi* in TCM has been described to be related to energy metabolism, in which ATP plays an important role.[3] During respiratory metabolism, bioenergy in the form of ATP is generated via glycolysis, the citric acid cycle, and the ETC. The final step of the ETC provides the most ATP, ~90%, to the cell. Thus, it is reasonable to hypothesize that *Qi* might relate to the ETC in the human body and *Qi* tonics, for treating *Qi* deficiency, might exert their effect on the process. Therefore, the mouse model of *Qi*-deficiency in TCM was used here to improve the understanding of the relationship between electric current generation and low energy metabolism of cells.

METHODS

Materials and reagents

All chemicals used in experiments were at analytical grade (China). Hepes was purchased from Yuanye Bio-Technology Co., Ltd. (Shanghai, China); collagenase IV and percoll from Biosharp Co., Ltd. (Heifei, China); the protonophore 2,4-dinitrophenol (DNP) and ATP inhibitor resveratrol (RVT) from Sigma-Aldrich, Inc. (St. Louis, MO, USA). Fifty milligrams of RVT or DNP were dissolved into 10 mL of ethanol to obtain 5000 ppm stock solutions. Then, 2 mL of each stock was diluted to 10 mL using perfusion buffer to produce 1000 ppm solutions.

Preparation of buffers and Chinese medicine

Perfusion buffer concentrate (PBC): NaCl (103.75g),