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Oxygen consumption of human heart cells in monolayer culture

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ABSTRACT

Tissue engineering in cardiovascular regenerative therapy requires the development of an efficient oxygen supply system for cell cultures. However, there are few studies which have examined human cardiomyocytes in terms of oxygen consumption and metabolism in culture. We developed an oxygen measurement system equipped with an oxygen microelectrode sensor and estimated the oxygen consumption rates (OCRs) by using the oxygen concentration profiles in culture medium. The heart is largely made up of cardiomyocytes, cardiac fibroblasts, and cardiac endothelial cells. Therefore, we measured the oxygen consumption of human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs), cardiac fibroblasts, human cardiac microvascular endothelial cell and aortic smooth muscle cells. Then we made correlations with their metabolisms. In hiPSC-CMs, the value of the OCR was 0.71 ± 0.38 pmol/h/cell, whereas the glucose consumption rate and lactate production rate were 0.77 ± 0.32 pmol/h/cell and 1.61 ± 0.70 pmol/h/cell, respectively. These values differed significantly from those of the other cells in human heart. The metabolism of the cells that constitute human heart showed the molar ratio of lactate production to glucose consumption (L/G ratio) that ranged between 1.97 and 2.2. Although the energy metabolism in adult heart *in vivo* is reported to be aerobic, our data demonstrated a dominance of anaerobic glycolysis in an *in vitro* environment. With our measuring system, we clearly showed the differences in the metabolism of cells between *in vivo* and *in vitro* monolayer culture. Our results regarding cell OCRs and metabolism may be useful for future tissue engineering of human heart.
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1. Introduction

Heart failure is a major cause of morbidity and mortality [1]. Despite progress in pharmacological and surgical therapies and device-based treatment, the survival rate of patients with severe heart failure remains poor. For severe heart failure, heart transplantation has been the only treatment, until now. The transplantation procedure is associated with various problems, including limited donor supply, the prolonged need for immunosuppression, and the risk of organ rejection [2]. In contrast, regenerative therapy has the potential to treat severe heart failure without the complications of transplantation. Recent research has shown that various heart tissue engineering methods used in regenerative therapy offer potential new approaches in the treatment of heart failure [3]. Further progress in the field of cardiovascular regeneration therapy requires the development of an efficient oxygen supply system for cardiac cells, because those cells require more oxygen for growth and maturation during culture. In order to develop such a system, it is very important to study the oxygen consumption and metabolism of cardiac cells in culture. Cellular viability and proliferation [4], protein synthesis [5], and carbohydrate metabolism [6] have all been reported to change according to the pericellular oxygen concentration. Therefore, the metabolism and function of cells is crucially dependent on the local oxygen environment. Several authors have reported on measurements of cellular oxygen consumption under *in vitro* culture conditions. For example, OCRs of renal mesangial and hepatic epithelial cells were estimated by measuring the pericellular oxygen concentration in a monolayer culture [7]. The OCR of mammalian cardiomyocytes was also measured in a monolayer culture which showed that oxygen consumption of beating cardiomyocytes was higher, by approximately 50%, than that of non-beating cardiomyocytes [8]. In another report, the oxygen concentration and cell viability within an engineered cardiac construct, based on neonatal rat cardiomyocytes cultured on collagen scaffolds, were measured

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and found to decrease linearly with the distance from the construct surface [4].

In measuring the OCR of cardiomyocytes in culture, animal cells have commonly been used as substitutes for human cells [9]. The establishment of hiPSC-CMs has made it possible to culture beating human cardiomyocytes *in vitro*, and to use them for specific experiments. In several recent papers, the OCR and extracellular acidification rate (ECAR) of hiPSC-CMs seeded on a specialized plate were measured simultaneously by using an extracellular flux analyzer to estimate the OCR/ECAR ratio to assess the relative contribution of glycolysis and mitochondrial respiration in energy generation [10,11]. In these studies, the OCR was measured for the purpose of high throughput screening, and thus lacked any detailed quantitative comparisons between different cell types. The aim of the present study was, therefore, to conduct a kinetic analysis of the OCRs of different cardiac cell types.

We developed an oxygen measurement system equipped with an oxygen microelectrode sensor. The system was used to measure the dissolved oxygen concentrations of culture media in a dish with a micro-scale. We were able to estimate cellular oxygen consumption based on the local oxygen concentration profile and thereby investigate the correlation between metabolism and oxygen consumption. These findings are expected to provide useful information to aid in the future advancement of cardiac tissue engineering.

2. Materials and methods

In this study, all procedures using animals were performed in accordance with the guidelines outlined by the Institutional Animal Committee of Tokyo Women's Medical University.

2.1. Cell culture

Human induced pluripotent stem cells (hiPSCs,253G1) [12], which were provided by RIKEN BRC (Tsukuba, Japan) through the National Bioresources Project of MEXT, Japan, were cultured, and hiPSCs-derived cardiomyocytes (hiPSC-CMs) were obtained using the bioreactor system, developed in our laboratory, as previously described [13]. Immunocytochemistry showed that approximately 80% of the cells were positive for cardiac troponin T. Following cardiac differentiation, the cells were dissociated with 0.05% trypsin/EDTA. By using a strainer (BD Bioscience, San Jose, CA), the cell aggregates were removed and single cells were reseeded at a density of 5.2×10^4 cells/cm^2. HiPSC-CMs were cultured in high-glucose Dulbecco's modified Eagle's medium (DMEM, Sigma, St. Louis, MO), supplemented with 10% FBS, and 1% penicillin–streptomycin (PS). Meanwhile, human cardiac fibroblasts (HCF, PromoCell, Heidelberg, Germany) were cultured in fibroblast growth medium (FGM 3, PromoCell), the cells were seeded at 5×10^3 cells/cm^2. Human cardiac microvascular endothelial cells (HMVEC-C, Lonza, Basel, Switzerland) were cultured in endothelial cells growth medium (EGM-2MV, Lonza), the cells were seeded at 5×10^3 cells/cm^2. Human aortic smooth muscle cells (HASMC, Kurabo, Osaka, Japan) were cultured in smooth muscle cell growth medium (HuMedia-SG2, Kurabo), the cells were seeded at 2.5×10^3 cells/cm^2. We also measured the OCR and metabolism of rat cardiomyocytes (rat-CMs) because they are frequently used in tissue engineering of heart. Rat-CMs were isolated from the hearts of 1-day-old Sprague–Dawley rats and were cultured as described in our previous report [14]. Isolated cardiomyocytes were cultured in a medium containing 6% fetal bovine serum (FBS), 40% Medium 199 (Invitrogen), 0.36% PS, 3.0 mM glucose, and 54% balanced salt solution containing 116 mM NaCl, 1.0 mM NaH_2PO4, 0.8 mM $MgSO_4$, 1.2 mM KCl, 0.8 mM $CaCl_2$, and 26.2 mM $NaHCO_3$. The isolated cardiac cells were seeded at a density of 2.5×10^5 cells/cm^2. Each type of cell was cultured as a monolayer in 35 mm-diameter dishes and maintained at 37 °C in humidified air containing 5% CO_2. The hiPSC-CMs were cultured for 7 or 8 days before oxygen measurement, while the other cell types were grown to confluence. The medium was changed every 2 or 3 days, and again 1 day prior to the oxygen measurement. We confirmed that hiPSC-CMs and rat-CMs were beating just before the oxygen measurements.

2.2. Measurement of the oxygen concentrations

As shown in Fig. 1, the oxygen concentration measurement system was composed of a microelectrode, a Clark-type oxygen microsensor with a 8–12 μm-diameter tip made of fragile glass (OX-10, Unisense, Denmark) connected to a sensitive picoammeter (PA2000, Unisense), *X*-, *Y*- and *Z*-axes linear actuators (Unisense) for manipulating the sensor, and a high-precision electronic balance (HTR-220, Shinko Denshi, Japan) for detecting the position of the tip of the electrode where came in contact with the bottom of the dish. The environmental conditions during measurement were maintained in a glove box hypoxia workstation ($INVIVO_2$ 300, Ruskinn Technologies), with a humidified atmosphere (with 65–80% humidity) containing 21% oxygen and 5% CO_2, maintained automatically. The position of the electrode and the signal from the

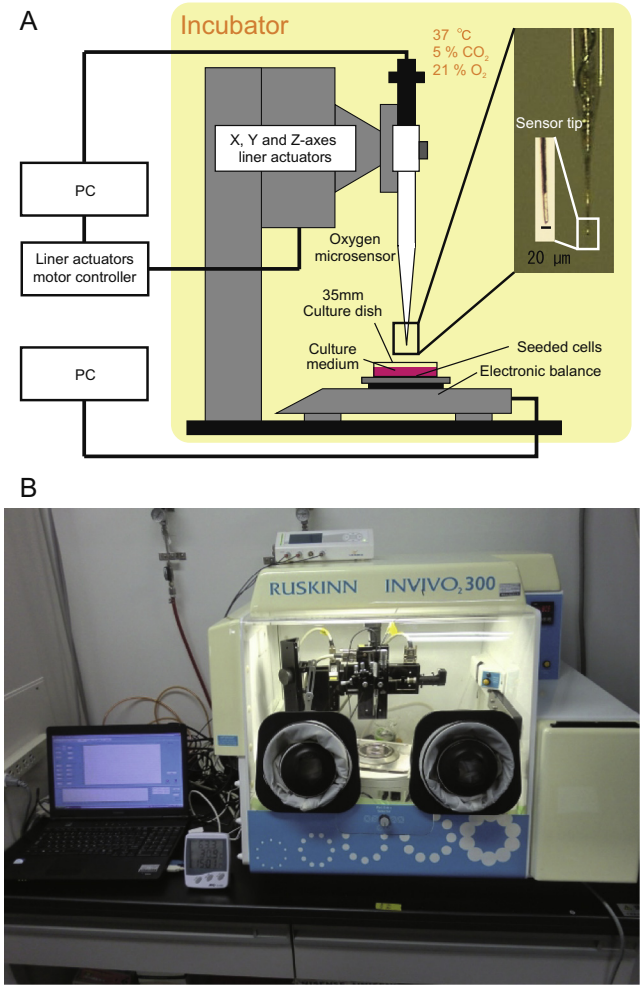

Fig. 1. Developed oxygen measurement system. The schematic illustration (A) and the over-view (B) of the system. See Section 2 for the details.

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