Available online at www.sciencedirect.com
SCIENCE DIRECT®
Sensors and Actuators B 108 (2005) 597–602
SENSORS AND ACTUATORS B CHEMICAL
www.elsevier.com/locate/snb

Metabolic and enzymatic activities of individual cells, spheroids and embryos as a function of the sample size

Hitoshi Shiku[a,*], Yu-suke Torisawa[a], Airi Takagi[a], Shigeo Aoyagi[b], Hiroyuki Abe[c], Hiroyoshi Hoshi[d], Tomoyuki Yasukawa[a], Tomokazu Matsue[a,*]

[a] *Graduate School of Environmental Studies, Tohoku University, Aramaki 07, Sendai 980-8579, Japan*
[b] *Hokuto Denko Co., Atsugi, Kanagawa 243-0181, Japan*
[c] *Tohoku University Biomedical Engineering Research Organization (TUBERO), Sendai 980-8575, Japan*
[d] *Research Institute for the Functional Peptides, Yamagata 990-0823, Japan*

Received 12 July 2004; received in revised form 27 November 2004; accepted 1 December 2004
Available online 11 January 2005

Abstract

Respiration, photosynthesis and peroxidase activities of living spherical samples, such as algal protoplasts, breast cancer spheroids and bovine embryos, were characterized with scanning electrochemical microscopy (SECM). The concentration profile of the metabolic product around the spherical sample was directly measured by scanning with a probe microelectrode. According to the spherical diffusion theory, the total mass transfer rate per spherical sample is linear to the multiplication of the sample radius and the concentration difference between the sample surface and the bulk of the solution. Therefore, the sample radius is a key parameter to assess the viability of the living samples. For example, we investigated the respiration and photosynthesis activities as a function of the size of the protoplast (*Bryopsis plumosa*). The respiration rate was linear to the cube of the sample radius. On the contrary, the photosynthesis rate was linear to the square of the sample radius, suggesting that the former is controlled by the volume of the protoplast, and the latter is controlled by the surface area of the protoplast. We will also discuss the size-dependent activity of the breast cancer spheroids and the bovine embryos. Furthermore, relations between the sample size, the concentration difference, and the oxygen consumption rate of the cryo-preserved bovine blastocysts were investigated.

Keywords: Scanning electrochemical microscopy; Respiration; Peroxidase; Spheroid; Bovine embryo; Cryo-preservation

1. Introduction

Scanning electrochemical microscopy (SECM) [1] has been successfully applied to investigate various biological systems including DNA [2], enzymes [3–5], antigen–antibodies [6–9], tissue [10–15] and cells [16–23], because of its non-invasive nature to quantitatively characterize localized chemical reaction under physiological conditions. Focusing on studies pertaining to the estimation of cellular activities, it appears that activities of the living samples show a significant diversity depending on the cell types and environmental conditions. In this study, we summarize the metabolic and enzymatic activities of various living samples and discuss the size-dependency of the mass transfer rate in each system.

SECM measurement visualizes the concentration profile around the sample; thus, the concentration difference between the sample surface and the bulk of the solution (ΔC) is the parameter that can be directly measured to quantitatively estimate the mass transfer rate. According to the spherical diffusion theory, the F value is given by the multiplication of ΔC and the sample radius (r_s) [24]. Subsequently, it can be easily assumed that the sample with a larger r_s value will have a higher metabolic activity. Besides there is still a need for the SECM measurement to more precisely characterize the quality of the bovine embryos [25] or the anticancer drug

* Corresponding authors. Tel.: +81 22 217 6167 (H. Shiku).
E-mail addresses: shiku@bioinfo.che.tohoku.ac.jp (H. Shiku), matsue@bioinfo.che.tohoku.ac.jp (T. Matsue).

0925-4005/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.snb.2004.12.030

sensitivity test using human breast cancer cells [26]. In the present study, we report other results that characterize the cryo-preserved bovine blastocysts. The quality of the frozen and thawn bovine blastocysts was judged according to the size, the ΔC value, and the mass transfer rate (F) of the single embryos and a detailed comparison of these blastocysts were carried out.

2. Experiment

2.1. Sample preparation

The preparations of protoplasts of the marine algae *Bryopsis plumosa* [4] and spheroids of a human breast cancer cell line (MCF-7) [26,27] were referenced from literatures. MCF-7 was donated by the Cell Resource Center for Biomedical Research (Tohoku University). In vitro maturation and fertilization of bovine oocytes and in vitro bovine embryo culture were described in other literatures [28]. Blastocysts on day 8 after fertilization were exposed to a freezing medium (containing 10% glycerol, Research Institute for the Functional Peptides, Yamagata, Japan) and loaded into 0.25 mL straws divided by air bubbles from the other two columns of the freezing medium. The straws were cooled using a program freezer (Tokyo Rikakiki Co. Ltd., MPF-1000). They were pre-cooled to −6 °C for 15 min, and then cooled from −6 to −30 °C at a rate of 0.3 °C min^{-1}. The straws were maintained at −30 °C for 120 min, and then stocked into liquid nitrogen. Thawing was performed in warm water at 37 °C and the embryos were then decanted into IVMD101 (Research Institute for the Functional Peptides, Yamagata, Japan) and incubated at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air.

2.2. Estimation of the metabolic rate of single spherical samples

All the samples (protoplasts of *B. plumosa*, spheroids of a human breast cancer cell line (MCF-7), and bovine embryos) were sufficiently sphere-shaped to be analyzed using the spherical diffusion theory. The mass transfer rate (F, mol s^{-1}) of a spherical sample can be calculated using the equation $F = 4\pi r_s D \Delta C$ [4,24,25], where D is the diffusion coefficient, ΔC is the concentration difference between the bulk and the sample surface ($\Delta C = |C^* - C_s|$), and r_s is the sample radius. C^* and C_s are determined from the intercepts at $r_s/(r + r_s) = 0$ and 1 of the $C(r)$ versus $r_s/(r + r_s)$ plot. (See Fig. 2(e) as example for bovine embryo samples.) The tip was scanned back and forth three times to estimate the average (AV) ± standard deviation (S.D.) ($n = 6$) of the ΔC for each sample. The r_s for the bovine embryo is defined as the embryo radius including the zona pellucida with a thickness of 3–20 μm.

The concentration profiles of oxygen (O_2), ferriceniummethanol (FMA^+), and ferrocenylmethanol (FMA) were measured by a scanning electrochemical microscopy (SECM) probe of a Pt-microelectrode at −0.5, +0.1 and +0.5 V versus Ag/AgCl, respectively. The single SECM measurement procedure takes 65 s with a tip scan rate of 14.7 μm/s. The measuring solutions for *B. plumosa*, MCF-7, and bovine embryos were seawater (at room temperature), HEPES buffer (at room temperature), or RPMI1640/HEPES (Gibco, at 37 °C), and IVMD101 (Research Institute for the Functional Peptide, Yamagata, Japan, incubated at 37 °C in 5% CO_2 and 95% air), respectively. The D value for each experimental condition is as follows: $D_{(oxygen)} = 2.18 \times 10^{-5}$ cm^2 s^{-1} (at 37 °C); $D_{(oxygen)} = 2.10 \times 10^{-5}$ cm^2 s^{-1} (at room temperature); $D_{(FMA)} = D_{(FMA^+)} = 7.00 \times 10^{-6}$ cm^2 s^{-1}(at room temperature). The bulk concentration (C^*) of oxygen in seawater (at room temperature), HEPES buffer (at room temperature) and IVMD101 (Research Institute for the Functional Peptide, Yamagata, Japan, at 37 °C) are 0.204, 0.210 and 0.209 mM, respectively. The POD activity of the algal protoplasts was measured in the seawater containing 0.5 mM FMA and 0.5 mM hydrogen peroxide. In this study, peroxidase (POD) activity is defined as the rate of hydrogen peroxide-dependent FMA^+ production and FMA accumulation by a single algal protoplast.

3. Results and discussion

3.1. Diversity of the metabolic activity as a function of the sample size

Fig. 1 shows the relation between the metabolic activity and sample size for various spherical living samples. Respiration and photosynthesis activities can be expressed as oxygen consumption and production rates, respectively. POD activity was quantified as FMA accumulation and FMA^+ production in the presence of hydrogen peroxide. The metabolic activities can be expressed on the Y-axis as F (mol s^{-1}) for all the studied cases. The slope (x) of the log(F, mol s^{-1}) versus log(r_s, μm) plot was determined from the line of least squares to clearly discuss the relation between F and r_s. The lines that best fitted with r_s^x were then over layered in Fig. 1.

Fig. 1(a) indicates respiration (filled circle) and photosynthesis activity (open square) as a function of the radius of the algal protoplast. The slopes of the log(F, mol s^{-1}) versus log(r_s, μm) plot for respiration and photosynthesis were 2.6 and 2.2, respectively. Generally, the F value should be linear to r_s^3 when the metabolic reaction is controlled by the volume of the sample. In the case wherein the reaction is controlled by the surface area of the sample, the F value should be linear to r_s^2. Although the plot tended to be variable, the slope for the respiration was clearly large when compared with that for the photosynthesis. POD activity of the same algal protoplast has been investigated and is shown in Fig. 1(b). For measuring the POD activity of the *B. plumosa* protoplast, 0.5 mM ferrocenylmethanol (FMA) and 0.5 mM hydrogen peroxide were added to the measuring solution. The reaction involv-